The myth of communities

Determining ecological quality of surface waters using macroinvertebrate community patterns

Rebi Nijboer

ALTERRA SCIENTIFIC CONTRIBUTIONS 17

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Determining ecological quality of surface waters using macroinvertebrate community patterns

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Contents

Abstract

Macroinvertebrate community patterns are often analysed and related to the ecological quality or conservation value of a water body, using an ecological typology or assessment system. The goal of this study was to determine the effect on the development or application of a typology or assessment system of different choices concerning (1) taxonomic adjustment of the data, (2) the data that are included in the analyses and the community variables focussed on, and (3) the techniques chosen.

It appeared that it is very difficult to distinguish and characterise macroinvertebrate communities unambiguously. In developing a typology or assessment system the results depend on the taxonomic level used and on the completeness of the data. Also, the choice of the technique influences the final result. In applying a typology or assessment system it is of importance to use the same taxonomic level as the data used for development and to include all taxa collected. Even taxa with low abundances or small distribution ranges appeared to be important. The number of rare taxa was indicative for a high ecological quality, in contrast to the total number of taxa in a sample. Communities composed of a high number of characteristic species were easier to distinguish than communities composed of more generalists.

In conclusion, community analysis is not an objective process, because of the large effect of small technical changes. This is also caused by the fact that any classification of species assemblages is artificial. A community only exists of a combination of populations at a moment on a site and it is impossible to collect the complete community in a sample. Communities are a continuum along an environmental gradient. Species are adapted to their environment (which is characterised by stability, favourability and impairment) by their life tactics. Therefore, water management should rather focus on these life tactics in relation to the environment to investigate the stressor(s) present and the possibilities for restoration. To establish the conservation value of a site, the number of rare species can be used.

Keywords: macroinvertebrate, assessment, typology, biodiversity, conservation, clustering, ordination, metric, data analysis, taxonomic resolution, rare species, indicator

Voorwoord

In mijn eerste jaren bij Alterra heb ik binnen het programma 'Aquatische Ecologie en Visserij' (programma 324, DWK, LNV) gewerkt aan het ontwikkelen van macrofauna-beoordelingssystemen voor oppervlaktewateren. Aangezien eerder goede ervaringen waren opgedaan met het gebruik van planten- en macrofauna-levensgemeenschappen leek deze benadering het meest geschikt. Ik liep daarbij echter steeds tegen het probleem aan dat de resultaten sterk konden veranderen als gevolg van een andere analyse methode. Daarbij was er geen maat om te beoordelen welk resultaat nu het beste was. Hierdoor is het idee ontstaan om in een proefschrift de beperkingen van de levensgemeenschapsbenadering aan de orde te stellen. Omdat het gebruik en standaardisatie van beoordelingssystemen voor de ecologie in oppervlaktewateren een 'hot item' werd sinds de publicatie van de Europese Kaderrichtlijn Water in 2000 kon ik binnen twee EU projecten (AQEM en PAEQANN) verder voortborduren op de resultaten uit het LNV programma.

Naast financiering zijn gegevens onontbeerlijk. De waterbeheerders wil ik daarom bedanken voor het aanleveren van gegevens van duizenden macrofaunamonsters. Zonder hen waren al deze analyses niet mogelijk geweest. Daarbij heb ik van hen in de wandelgangen veel geleerd over de problemen in de dagelijkse praktijk van het waterbeheer.

Mijn copromotor Piet Verdonschot heeft mij ingewijd in de macrofauna, de data-analyse en het wereldje van de aquatische ecologie en het waterbeheer. Hij was zelf een grote aanhanger van de levensgemeenschapsbenadering. Gelukkig stond hij ook open voor mijn argumenten en ideeën. De vele leuke discussies hebben geleid tot het idee voor dit proefschrift en bijgedragen aan de verdere uitwerking. Piet bedankt voor je positieve, kritische houding!

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Maar ook de link met de maatschappij en de politiek is belangrijk. Hiervoor heeft Kees Verhaar gezorgd. We voerden gesprekken over wetenschap, landbouw, sociologie, ecologie en politiek, waarvan we beide veel geleerd hebben. Dat onder het genot van goede muziek en heerlijk eten. Dank je wel Kees!

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Rink Wiggers hebben me veel geleerd over macrofauna soorten, zodat het niet bleef bij beestjes op een floppy. Ik neem altijd graag een kijkje door de microscoop als er weer eens een bijzondere vondst is gedaan. Ik hoop dat jullie nog eens een nieuwe soort vinden! Mijn kamergenootje Hanneke Vlek wil ik bedanken voor haar gezelligheid zowel op Alterra als in de hotelkamers die we gedeeld hebben. Ook zij liep vaak tegen dezelfde problematiek aan als in dit proefschrift beschreven is. Ik hoop dat je een mooi vervolg proefschrift gaat schrijven! De rest van het team ben ik dankbaar voor de goede sfeer waarin iedereen gewoon zichzelf kan zijn.

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Menko, ik ben heel blij dat ik jou heb leren kennen en dat het proefschrift toen al af was!

1 Introduction

Ecological assessment

The goal of this study was to determine the effect of choices made during data analyses on the results of an ecological typology or assessment system for surface waters. Hydrological changes, physical disturbance and point and non-point sources of pollution are examples of factors responsible for a broad-scale deterioration of aquatic ecosystems (Petts, 1990; Boon, 1992; Kristensen & Hansen, 1994; Verdonschot, 2000). Growing concern for public health and the worsening quality of the environment asked for the development of systems to monitor and assess the quality of surface waters (Armitage *et al.*, 1992). To restore surface waters, managers need techniques to identify the present status of the ecosystem and to predict what changes they can expect if the environment changes either due to degradation or to restoration measures.

The development of systems for the assessment of the quality of fresh waters has a long history (reviews: Metcalfe, 1989; Verdonschot, 2000). The first systems to evaluate the quality of water bodies were based on chemical or physical measurements. Later, the biotic aspect got more attention. Karr (1991) stressed that species can react to a complex of factors. That is why bio-indicators are more appropriate for assessment than just chemical or physical measurements, as they are integrators of a complex of environmental circumstances.

In the first biotic assessment systems, indicator species were used (e.g., in the saprobic system of Kolkwitz & Marsson (1908, 1909)). These systems were often based on the tolerance of species, genera or families for pollution (Armitage *et al.*, 1992). Based on these indices classification systems were designed (Warren, 1971). These early systems included the influence of a single environmental factor on, different species, e.g., the 'Trent biotic index' (Woodiwiss, 1964). For a long time, biological assessment of water quality had been uni-dimensional (Cairns and Prett, 1993) and focussed for example on organic pollution.

However, species always respond to a complex of environmental variables and they can also influence each other, for example by competition. Therefore, the use of one species as indicator has its shortcomings (Karr, 1991). During the last decades, ecological assessment systems have been developed. This was stimulated by the development of integrated ecological indices (Karr *et al.*, 1986; EPA, 1988). Such systems are based on a combination of indicative variables, e.g., species richness, or the number of species of indicative groups, for example, the number of Ephemeroptera, Plecoptera, and Trichoptera species (Lenat, 1988). Indices, such as the Biological Monitoring Working Party (BMWP) scoring system (Armitage *et al.*, 1983) and rapid

bioassessment techniques (Karr, 1981; Karr *et al.*, 1986; Plafkin *et al.*, 1989) have become very popular.

Ecological assessment systems focus on assessing the ecological quality of a water body, which is the quality of the total of environmental conditions reflected by the species composition (Verdonschot, 1990). In ecological assessment systems species of several higher taxa are used e.g., all macroinvertebrates in RIVPACS (Wright *et al.*, 1993) and EKOO (Verdonschot, 1990) together with a high number of environmental variables.

In the ideal situation the whole community present at a site should be considered in an assessment system. However, it is impossible to include all different kind of organism groups in one system, because sampling methods, the type of data revealed, and the type of analyses each group needs are too different. The group of benthic macroinvertebrates has been used in many assessment systems. They are recognised as one of the most reliable biological indicator groups in aquatic ecosystems (Hellawell, 1986). They play a key role in food web dynamics, linking producers and top carnivores, and a number of species have clear responses to environmental variables. Their spatial sedentariness and intermediate life span, from several months to several years, make macroinvertebrates ideal as for an integrative and continuous indicator group of water quality (e.g., Sládeček, 1973; Hellawell, 1986). Therefore, this study will focus on macroinvertebrates.

Nature conservation and biodiversity

An ecological assessment system can also be used for assessing the conservation value of water bodies. There are four main aims of assessing the conservation value of rivers (Boon, 2000a), which can be extrapolated to other water types:

a. To conserve representative examples of all major river types within a defined geographical area;
b. To conserve rare or threatened riverine species, or examples of rare or threatened habitats;
c. To devise appropriate management strategies for specific rivers based on a broad assessment of their conservation value;
d. To ensure sustainability in the ecological structure and function of all rivers.

Since the Convention on Biological Diversity has been signed by many countries at the UN conference on environment and development in Rio de Janeiro in 1992, biodiversity has become a popular issue in nature conservation. The Convention on Biological Diversity gives a formal definition of biodiversity in its article 2: 'biological diversity means the variability among living organisms from all sources including, *inter alia*, terrestrial, marine and other aquatic ecosystems and the ecological complexes of

which they are part; this includes diversity within species, between species and of ecosystems'.

Conservation of biodiversity should focus on three levels: conservation of habitats, conservation of species and conservation of genetic diversity. However, the high profile of biodiversity issues has led to an undue focus on species richness, under the misapprehension that the word biodiversity is synonymous with species richness and that rivers with high species richness are therefore necessarily important for conservation and vice versa (Boon, 2000b). This also implies that the word biodiversity is often used to value the importance of a single water body, while in fact the spatial scale at which biodiversity should be concerned is much larger.

Because systems to assess the ecological quality of surface waters already had a long history before the biodiversity concept became popular, most of these systems do not focus on assessing the conservation value of a water body. Many ecological assessment systems focus on dominant or common species. However, from the perspective of nature conservation or conservation of biodiversity the special species, for example, the threatened or rare species or the species that are indicative for undisturbed conditions should be highlighted (Boon, 2000a).

Developing and using an ecological assessment system

The main steps in developing an ecological assessment system are illustrated in Fig. 1. First, sampling sites in a series of water bodies are selected. The quality of the sites varies from reference conditions to the most impacted sites. In the field, physical and chemical variables are measured (for an example of which variables and methods: Verdonschot, 1990) and a water sample is taken for analysing the chemical constitution of the water. Next, the macroinvertebrate community composition in these water bodies is determined by sampling the habitats within the water body. From each habitat a replicate sample is collected. In most cases a multihabitat sample is taken. This means that each habitat is sampled in proportion to its presence and all habitats are combined into one sample. The sample is taken to the laboratory and the animals are sorted after sieving the sample. The animals are then identified. The numbers of individuals per taxon are put into a database for each sampling site.

First, the data are used to classify the water bodies, because relations between degradation and macroinvertebrates communities differ between water types (Verdonschot, 1990). Subsequently, multivariate or metric calculations are carried out to establish the communities present and the relation with environmental variables and the quality status of the water body.

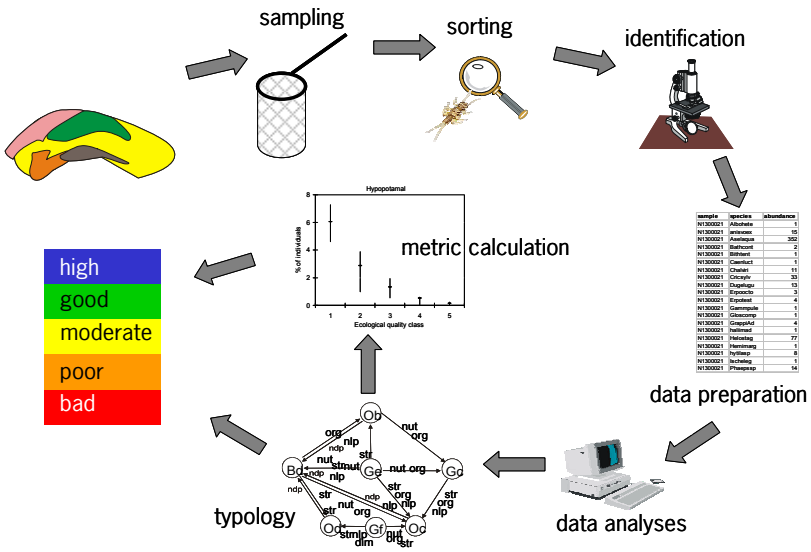

Fig. 1.1. Steps to be taken from sampling to ecological assessment system.

With multimetrics (Barbour & Yoder, 2000), different community characteristics (indices) are combined, for example the ratio between grazers and filterers or the saprobic index, which indicates the extent of organic pollution using species indications for saprobic classes. Many combinations of indices are possible. Indices are either based only on the distribution of groups of species or also on knowledge (from earlier research or literature) about the ecological preferences of the species.

Multivariate analyses (Jongman *et al.*, 1995) often consists of two main types of analyses: (1) classification of the sites into site groups (interpreted as community types) using clustering techniques in which samples with similar species composition are clustered and (2) ordination to relate the sites (or the site groups) to the explanatory environmental variables. The result of multivariate analyses is in most cases an ecological typology in which community types are characterised by their species composition and environmental conditions.

Finally, the sites or site groups have to be given a value to make the system useful for quality assessment purposes. This is often done by distinguishing five ecological quality classes as required by the Water Framework Directive (European Commission, 2000). Therefore, the metric results (more often combined in a multimetric) are divided into five ranges, whereby each range indicates one of the five ecological quality classes. In multivariate analyses the ecological quality classes are assigned to the site groups interpreting the species composition and the ranges of environmental variables of the sites.

Water managers can apply an ecological assessment system to evaluate the ecological quality of a water body. Therefore, the site should be sampled, the animals sorted and identified. The data are put into the assessment system and the ecological quality class is then calculated (Hering *et al.*, 2004).

The quality of assessment systems

Each step in the methodological chain has its effect on the overall result, thus on the quality of the ecological typology or assessment system and on the result of the classification or assessment of a water body. The goal of this study was to determine the effect of choices made during data analyses on the results of the typology or assessment system. The first steps of the process, sampling, sorting, and identification of the animals, are not included because this research was carried out with data collated from water district managers. Therefore, parts concerning sampling, sorting and identification methods were already fixed.

The research focused on three aspects of developing and using an ecological assessment system. First, the effect of the quality (completeness) and preparation of the data set was studied. Second, different techniques of analysing the data to develop an assessment system were compared. Third, it was studied which community variables should be included in the analyses to develop an assessment system. In applying an existing assessment system, the same technique is used as was used for development but it is possible to experiment with the community variables and the completeness of the data to be used. For short, it was tested whether the results of the development and use of typologies and assessment systems differ depending on:

a. The quality and type of data that are used and how they are prepared before analyses;
b. The community variables that are included in the analyses;
c. The technique that is chosen.

Quality of the data

A sufficient consistency of data used for the development as well as the use of typologies or assessment systems is a basic need. Data have to be equally detailed and collected in similar ways to be comparable. In many countries regular monitoring is done by different persons or institutions. In the Netherlands more than twenty water boards are responsible for monitoring the water bodies in their water management district. If data of different water managers are combined to carry out research on a national level, mutual inconsistencies may occur. Also, application of a typology or assessment system with data that are not comparable with the data used for development might be problematic.

The quality and completeness of data (how many of the species present at a site are collected and in representative numbers?) depends on the sampling and sorting strategy. Although water managers in the Netherlands have standardised some aspects of the sampling strategy, there are still many differences. Important aspects that have influence on the number of individuals and the species collected are: (1) the sampling season (Barbour *et al.*, 1992), (2) the surface area (Rodriguez & Wright, 1991; Marchant, 1990), the representativeness of the habitats present at the site, sampling depth (only sampling material of the bottom surface or including the upper bottom layer), and the way the pondnet is handled. During the sorting process the time used, experience, and number of people that is involved is of importance as well as the methods. Sometimes the whole sample is sorted, sometimes only a part. During sorting, small animals can be lost in particular, because of the use of sieves with mesh sizes too large to retain all the organisms (Dukerschein *et al.*, 1996) or by picking out only the larger individuals. Also the sorting method (e.g. sorting the whole sample or sorting a sub sample) influences the number of rare species collected. However, exact information about sampling and sorting methods was often lacking for the data used in this study. Therefore, this aspect was not included.

The next step after sampling and sorting the macroinvertebrates is the identification. The accuracy of the identifications differs between data sets. Systematics and taxonomy are important aspects in processing samples for the use in classification and ecological assessment of water bodies. Following Resh & McElravy (1993), species level identification is substantial because the species is to be seen as the basic biological unit with the highest information content and it increases sensitivity and detection of subtle changes in ecological quality assessment. In fact, the population of a species is the only real entity that is present at a site and has relationships with environmental variables and populations of other species. However, in many cases identification of all organisms to species level is not possible. Taxonomy of macroinvertebrates is complex and increased knowledge has only recently become widespread. Within the Netherlands, the level of identification differs between water district managers and is strongly dependent on the experience and specific interest of the personnel that identifies the specimens and on the identification keys used. Even if all identifications are carried out by one person, there can be differences between samples and inconsistent data sets can exist, e.g., caused by early larval instars that can only be identified to genus level whereas the later instars in the same data are identified to species level.

Identification at different levels results in inconsistencies within a data set. For example, in one sample the group of water mites is identified to species level and in another sample this group is identified only to genus level. For analyses, the data set should be consistent to ensure that results are unambiguous (Gauch, 1982; Jongman *et al.*, 1995). This means no taxa may overlap, because systematic overlap results in a multiplication of the same information in a sample. Systematic overlap can also result

in artificial difference between samples. Therefore, data should be taxonomically adjusted before analyses. The effect of taxonomic adjustment on classification of macroinvertebrate samples was studied in **Chapter 2**: 'Taxonomic adjustment affects data analysis: an often forgotten error'.

Community variables

A community is the assemblage of plants and animals in any given physical environment (Smith, 1986). Community structure or characteristics are often used to assess the ecological quality of water bodies. This is based on the assumption that in water bodies with similar environmental conditions similar species assemblages occur. Ecological data are multivariate, complex, bulky, and showing noise and redundancy (Gauch, 1982; Legendre & Legendre, 1998; Jongman *et al.*, 1995). Metrics as well as multivariate analyses, such as clustering and ordination are developed to extract patterns from ecological data and to describe communities, focussing on certain community variables. Techniques use different community variables but the user can also choose to focus on a certain community variable to reduce the complexity of the results or to make the analyses more efficient. Important community variables are:

a. The distribution and abundance of species (dominant, common, rare species can get different weights in calculations);
b. The total number of species;
c. The indicative value of the species (calculated by the technique or based on autecological databases).

Distribution and abundance of species are important variables in relation to the definition and use of dominant and rare species. The presence of rare species (species that have small distribution ranges, which means they are restricted to relatively few water bodies) can be important because they could be threatened and valuable for biodiversity conservation. Dominant (species with high abundances) or common species (species that occur in many of the water bodies sampled) are often used because they are regularly found and they may be important in the ecological functioning of the ecosystem. Many assessment systems use or depend on dominant and common species to assess the ecological quality.

The total number of species is often included in ecological assessment because it would support the biodiversity concept. Using the total number of species is based on the hypothesis that the number of species at a site decreases if degradation takes place.

Indicator species are often used because they are either directly related to one or more environmental variables or they indicate the community type that is present at a site. The use of indicator species might reveal a clear distinction of community types and ecological quality classes because the occurrence of these species may reflect the systems environmental conditions including human impact. The three community variables are discussed in more detail in the following sub paragraphs.

Distribution and abundance of species

The distribution range (frequency of occurrence) and abundance are often used to define dominant species (species with high abundances), common species (species that occur at many sites) and rare species (species that occur in low abundances or occur at few sites). Dominant and common species are often used in ecological assessment. However, it is a question whether the results using only dominant and common species are detailed enough. These species are often tolerant for many different environmental conditions and therefore occur in many different water bodies of different ecological quality. They do probably not sufficiently express the difference between community types and ecological quality classes. Differences for dominant and common species are found more often in abundances than in presence or absence of these species.

The occurrence of rare species may be more indicative for the ecological quality and the community type. In population and community ecology rare species are regarded as those species that have a low abundance and/or a small distribution range (Gaston, 1994). Both, abundance and distribution range criteria are also used to classify rarity of macroinvertebrates in water bodies. Species with low abundances and large distribution ranges are probably not very indicative for specific environmental conditions because a large distribution range implies that the species can survive over a variety of circumstances. Environmental variables could cause low abundances of a species, for example if species live under sub-optimal conditions in the outer range of their geographical distribution area. Often, species that always occur in low numbers have biological traits that prevent a fast population growth, e.g., a long life cycle, few offspring, dependence on a host species, or dependence on nutrition sources (e.g., predators seem to be rarer than prey species, as was stated by Spencer, 2000). Sometimes, species have low abundances at the outer ranges of their geographical distribution area.

Species that are limited to a small range of water bodies may be more indicative for the environmental conditions and/or ecological quality of their environment. They can be rare because they are geographically restricted or require specific habitat conditions, which are rare (Gaston, 1994; Lawton, 1999). For this group, biological traits might be important as well, e.g., a low dispersion and colonisation capacity or required interactions with other species (Gaston, 1994). Species that occur in only few water types or habitats can have a high abundance at the sites where they occur. Species with a small distribution range in combination with low abundances can be vulnerable, and therefore important for conservation of biodiversity. Especially, rare species that have decreased because of human disturbance or rarity of their habitat have a greater chance to become extinct in a region. Rare species are important for conservation of biodiversity and they can be useful in ecological assessment, especially

those rare species that are indicative for a high ecological quality. Probably, the number of rare species at a site is an indicative variable in ecological assessment.

Before the number of rare species can be used a list of which species are rare is needed. **Chapter 3**: 'Rare and common macroinvertebrates: definition of distribution classes and their boundaries' describes the criteria used for creating a list of the distribution classes (from very rare to abundant) for Dutch macroinvertebrates. In **Chapter 4**: 'The effect of excluding taxa with low abundances or taxa with small distribution ranges on ecological assessment', rare species are defined either on abundance or on distribution criteria. Both types of rare species are related to autecological characteristics and ecological quality classes. In **Chapter 5**: 'Ecological assessment of aquatic ecosystems: Taxa richness is not the item, rare taxa are', the question whether rare species are related to habitat variables or ecological quality is further studied.

Excluding rare species from ecological assessment

In the ideal situation all the species at a site are sampled in the ratio that they are present at the site and are identified to the species level. However, this is not the reality. A sample includes only a biased part of the species present and during the sampling and identification process more specimens and even species are missed or get lost.

It is also possible that people choose to reduce the amount of biotic data to reduce costs. Processing complete macroinvertebrate samples implies high costs for sorting and identification of all specimens. To build assessment systems it is often recommended to use detailed information about the communities that are included, for example using complete samples and identification to species level. However, data sets with hundreds of species are hard to analyse and to interpret. For management purposes, it would be more effective if the number of species that is necessary as input for an assessment system could be reduced.

Rare species are the first ones that are lost or removed during processing of the sample, because of their low numbers (Cao *et al.*, 1998). Especially, in multivariate analysis it is common practice to exclude or downweight the rarest species in the data set (e.g., Boulton *et al.*, 1992). The usual rationale for adopting this approach is the assumption that the rare species in a sample are those that are present by chance, are not being true representatives of the community, and their inclusion will only add noise to the analyses (Gauch, 1982). This is also done for species that occur in a few samples, independent of their abundance. Others have excluding rare species because valuable information can be lost (e.g., Cao *et al.*, 1998; Karr and Chu, 1997). It is widely recognised that rare species constitute the majority of species diversity in many natural communities (Krebs, 1985). A major part of the dissimilarity between communities may frequently reside in the species composition of the rare

representatives. Rare species can be excluded during the development of a typology or assessment system or in the application with new data.

In **Chapter 6**: 'Comparison of clustering and ordination methods implemented to the full and partial data of benthic macroinvertebrate communities in streams and channels' the effect of excluding taxa with low abundances on development of a typology was tested.

The effects of excluding rare taxa on applying an assessment system were studied in **Chapter 4**: 'The effect of excluding taxa with low abundances or taxa with small distribution ranges on ecological assessment'.

Taxa richness

The number of species or taxa in a sample is often used as a criterion for assessment of ecological quality. Particularly, since biodiversity issues have got much more attention, species richness has become an indicator for biodiversity for many practical purposes (Larsen & Herlihy, 1998). In many scoring systems taxa richness is included. Many researches have shown taxa richness to be a good metric for environmental assessment studies, e.g., Resh (1994), Sandin and Johnson (2000), Resh *et al.* (2000). Rapport (1991) showed that human impact was indicated by reduced taxa richness.

However, some authors suggest that high taxa richness is not always related to unimpacted sites. Rivers with high species richness are not necessarily important for conservation and rivers with low species richness can be interesting (Boon, 2000b). Relatively natural rivers in some locations e.g., with harsh environments (Wright *et al.*, 1998) can be species-poor. It is probably hard to compare taxa richness between sites, because the number of taxa is dependent on many factors and it is also related to the water type and the type of human impact. It is still the question whether taxa richness can be related to the ecological quality of sites or to habitat variables.

Chapter 5: 'Ecological assessment of aquatic ecosystems: Taxa richness is not the item, rare taxa are', focused on the number of taxa in a sample and the relation to habitat variables and ecological quality.

Indicator species

Instead of using all the species found at a site to develop or use a typology or assessment system one can focus on the indicator species only. The selection of species as representatives of species assemblages or even whole communities or ecosystems differs from the traditional definition of indicators, in which one or a few indicators were chosen that indicated one specific variable, for example, organic pollution (Kolkwitz & Marsson, 1908, 1909). Community indicators only indirectly indicate the environment. They represent the community present at a site. The community is of course indicative for the complex of environmental variables as well as biological interactions. The major advantage of a community based approach is that

a whole complex of interacting environmental variables is included in the species grouping (e.g., Gauch, 1977; Boesch, 1977; Jongman *et al.*, 1995) and thus in the selection of indicator species. Indicator species are often selected using numerical techniques, based on the distribution and abundances of the taxa in the community types (site groups). Community indicators may be useful in ecological assessment systems. In the development of ecological assessment systems using only indicator species might be a possibility to make results less complex and to reveal direct relationships between communities and environmental conditions.

In **Chapter 6**: 'Comparison of clustering and ordination methods implemented to the full and partial data of benthic macroinvertebrate communities in streams and channels', the usefulness of indicator species in two techniques was studied.

Also, in applying a typology or ecological assessment system, indicator species could be used to reduce the large amount of data and work concerned with collecting species data. It would be very efficient for water managers if they could only focus on indicator species. The question is whether just a limited selection of species can sufficiently indicate the quality or characteristics of a whole community. Other possibilities for reduction of the large amount of species data is sorting and identifying only one taxonomic group, for example, the Odonata. The effect of using subsets of indicator taxa and taxa from one taxonomic group was tested in **Chapter 7**: 'Can indicator taxa be used as substitutes for freshwater macroinvertebrate communities?'

Techniques

Analysing community patterns is difficult because data sets are non-linear and composed of many species varying over different locations and time (Chon *et al.*, 2000), and with a different distribution and density. At a site, only part of the community present is collected at a certain moment. Therefore, each sample contains some information about the community but none is complete. Techniques can help to interpret the community structure. Multivariate techniques such as principal component analysis, cluster analysis, and correspondence analysis have been used to understand ecological data, to extract communities, and to relate these to the environment (e.g., Gauch, 1982; Jongman *et al.*, 1995; Legendre & Legendre, 1998; Ludwig & Reynolds, 1988). Assessment systems, such as EKOO (Verdonschot & Nijboer, 2000) and RIVPACS (Wright, 2000) are based on a stepwise progression of clustering and ordination. The basic unit of each typology and ecological assessment system is the community type. Community types are defined using classification of sites with similar species composition using clustering techniques. Next, community types are related to environmental conditions using e.g., ordination. Many techniques are available varying from classical clustering techniques (e.g., Van Tongeren, 1986) to new advanced techniques such as Artificial Neural Networks (e.g., Chon *et al.*, 1996).

In the ideal situation different techniques should result in a similar classification of samples. In which extent this is true was tested in **Chapter 6**: 'Comparison of clustering and ordination methods implemented to the full and partial data of benthic macroinvertebrate communities in streams and channels', in which two techniques were compared: clustering and ordination on the one hand, and Artificial Neural Networks on the other hand.

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2 Taxonomic adjustment affects data analysis: an often forgotten error

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Abstract

Macroinvertebrate community patterns are analysed using multivariate analyses, such as clustering and ordination. Identification to different taxonomic levels results in inconsistencies within a data set. But, for multivariate analyses, a data set should be consistent to ensure that results are unambiguous. Therefore, data should be taxonomically adjusted before analyses. In this study we tested the effect on the classification results using two methods of taxonomic adjustment: (1) to remove a genus/family from the data set if species within the genus or family occur as well and (2) to aggregate species to the genus or family if both species and higher level occur in the data set. Both data sets were analysed using the clustering program FLEXCLUS.

The results showed that taxonomic adjustment affected the classification results. The 'species' data set resulted in a higher number of clusters. The changes in classification results are not predictable. Clusters can be aggregated or split when species level is used instead of a higher taxonomic level. Both methods have disadvantages. Aggregating species to higher taxonomic level can result in loss of variation especially if the species within a group have different ecological preferences. Removing higher level taxa from the data can result in a loss of a high number of individuals. A solution may be to use both methods within one data set. The choice of the most suited processing method should be made per taxonomic group, based on the combination of occurrence, abundance and ecological relevance of the species within a taxonomic group. If species occur in many samples, numbers of specimens are reasonable, and species differ ecologically, they should be kept in the data set. Otherwise, aggregation is preferable.

Introduction

Macroinvertebrate assemblages are often used in water assessment and water management. (e.g., Armitage *et al.* 1983, Real *et al.* 1992, Johnson & Wiederholm 1989, Nijboer & verdonschot 1998). Macroinvertebrate taxa are grouped and related to environmental conditions. By including all taxa to describe assemblages inter-species relationships may be taken into account.

One of the techniques used for constructing assemblages is clustering analysis, whereby groups of samples (clusters) that have a comparable species composition are identified. Results of clustering are combined with ordination results to relate clusters to environmental factors (Verdonschot & Nijboer, 2000).

Table 1. Example of an inconsistent data set. The table shows the numbers of occurrences and individuals for the genus *Gammarus* and the family Tubificidae.

taxon	number of occurrences	number of individuals
***Gammarus* sp.**	**30**	**5360**
G. pulex	29	8347
G. fossarum	21	24770
Tubificidae	**38**	**8021**
Tubifex tubifex	16	2113
Potamotrix hammoniensis	3	145
Ilyodrilus templetoni	4	39
Aulodrilus limnobius	3	133
A. pluriseta	9	215
Rhyacodrilus coccineus	2	19

The data set, should be consistent to ensure that cluster results are unambiguous (Gauch 1982, Jongman *et al.* 1987). This means no taxa may overlap, because taxonomic overlap results in a multiplication of the same information in a sample. Taxonomic overlap can also result in artificial difference between samples. Table 1 shows an example of an inconsistent data set. Some specimens were identified to the genus *Gammarus* and other specimens were identified to *G. fossarum* and *G. pulex*. Both genus and species occur in more than one third of the samples. The same problem exists in the family Tubificidae, but in this group the species are less abundant.
Inconsistency of a data set can be caused by:

- Different sampling and sorting techniques;
- Seasonal variation;
- Early larval instars that are difficult to identify;
- Limited taxonomic knowledge about certain groups;

- Use of different identification keys;
- Availability of specialists for identification.

Inconsistent data need to be identified and eventually removed from the data set before they are analysed. Either the species or the higher taxon (genus or family) should be skipped if they are both present.

However, skipping higher taxa can lead to a loss of groups, especially in samples in which no species of that group are identified. Alternatively, aggregation of species to a higher level results in a less diverse data set and information loss if the species differ ecologically. In this study we investigated the effect of both methods (skipping genus/family or aggregating species) on the results of cluster analysis.

Methodology

Data processing

A data set consisting of 67 samples from different streams in the eastern part of the Netherlands was analysed. The data set contained 477 taxa. First the original data set was taxonomically processed. Two methods were used:

- Skip genus/family: If genus/family as well as species of a taxonomic group occurred, the genus/family was omitted and the species were kept. Groups that had been identified consistently (in which only species or only genus or family occurred) were retained. The result was a 'species' data set in which the number of higher taxa was reduced (363 taxa remained).
- Aggregate species to genus/family: If genus/family as well as species of a taxonomic group occurred, the species were aggregated to genus/family. The number of individuals of all species belonging to the respective genus or family in a sample was consolidated. The 'genus/family' data set included 249 taxa.

Clustering

Both 'species' and 'genus/family' data sets were clustered using the program Flexclus (Van Tongeren 1986). This program aggregates samples into groups with similar species composition. Depending on the chosen treshold value, the clustering results in more or fewer sample groups.

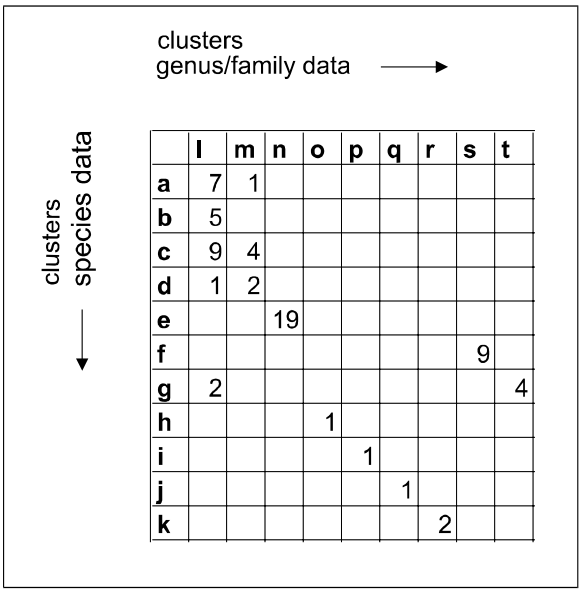
In the first clustering, a range of treshold values was used. Secondly, results from both data sets were compared by using the same treshold value. In this step, relocation of samples (Van Tongeren 1986) was carried out after the initial clustering. In the last step the clustering was repeated without some well-defined clusters that showed no difference between species and genus/family data. Before clustering, all abundances were transformed into Preston classes (Preston 1962, Verdonschot 1990).

Results

Table 2 shows the number of clusters that resulted from clustering of both data sets with different treshold values. The 'species' data set consistently resulted in more clusters.

Table 2. Number of clusters resulting from clustering with different treshold values.

treshold value	number of clusters 'species' data set	'genus/family' data set
0.05	6	3
0.10	7	6
0.15	11	7
0.20	13	9
0.25	16	11
0.30	22	13



	l	m	n	o	p	q	r	s	t
a	7	1							
b	5								
c	9	4							
d	1	2							
e			19						
f								9	
g	2								4
h				1					
i					1				
j						1			
k							2		

Fig. 1. Comparison of clusters resulting from 'species' versus 'genus/family' data (treshold value was 0.2). The number of samples occurring in both clusters is given in each cell.

To compare the cluster results, a treshold value of 0.2 was chosen. At this treshold value, cluster e in the species data was exactly the same as cluster n in the

genus/family data (Fig. 1). The choice of other threshold values did not change the composition of this cluster. This was also true for cluster k in species data (= cluster r in genus/family data) and some separate samples. Cluster f in species data (= cluster s in genus/family data), on the other hand, was split within the 'genus/family' data set at a higher treshold value. The samples within cluster f were included in the next clustering. Cluster e and k (species data) were easily recognised by the program in both cases. This means that the samples in these clusters were not only characterised by their species composition but also by genera or families. Several families were only present or only absent in these clusters.

Table 3. Occurrence and abundance of taxa in cluster b and cluster c. Grey cells include 'genus/family' data, white cells include 'species' data.

Taxon	cluster b		cluster c	
	% occurrence	mean Preston class	% occurrence	mean Preston class
Cricotopus sp.	85	5	33	2
C. sylvestris agg.	77	4	15	2
C. bicinctus	46	5	-	-
Erpobdellidae	100	7	100	4
Erpobdella octoculata	92	7	46	4
E. testacea	69	3	15	3
Dina lineata	23	1	-	-
Haliplus sp.	92	3	50	2
H. wehnkei	69	2	-	-
H. laminatus	62	2	8	1
H. ruficollis	31	3	15	2
H. heydeni	8	2	-	-
H. lineatocollis	23	1	8	1
H. fluviatilis	23	2	-	-
H. immaculatus	15	2	-	-

Clustering was repeated whereby species data clusters e, k and the separate samples were left out. The cluster results (treshold value 0.15) are shown in Fig. 2. Aggregation of species to genus or family resulted in two effects. Cluster b and c in the species data were aggregated into cluster h in the genus/family data. Cluster d in the species data was split and both parts of cluster d were aggregated with cluster h or cluster i in the genus/family data. Aggregation of clusters occurred because species information was lost (Table 3). The difference between the samples in cluster b and c was based on species and their abundances. The higher taxonomic units *Cricotopus* sp., Erpobdellidae, and *Haliplus* sp. occurred in both clusters after aggregation of species

to genus/family. Therefore, samples of clusters b and c were aggregated when 'genus/family' data were used.

Conversely, cluster d was split. In both parts species were not identified for the groups, which were omitted in the 'species' data set (Table 4). Thus, no difference occurred between samples when 'species' data were used. But, at genus/family level there were obvious differences. In half of the samples of cluster d, Tipulidae and Tubificidae occurred, but in the other half of the samples *Orthocladius* sp. was abundant. In this case, the 'genus/family' level contained more information than the 'species' data did.

Table 4. Occurrence and abundance of taxa in both parts of cluster d. Grey cells include 'genus/family' data, white cells include 'species' data.

taxon	cluster d part 1		cluster d part 2	
	% occurrence	mean Preston class	% occurrence	mean Preston class
Orthocladius sp.	-	-	100	5
O. subg.	-	-	33	6
O. thienemanni	-	-	-	-
Tipulidae	75	1	-	-
Tipula gr. oleracea	-	-	-	-
Tubificidae	100	8	-	-
Ilyodrilus templetoni	-	-	-	-
Aulodrilus limnobius	-	-	-	-
Aulodrilus pluriseta	-	-	-	-
Tubifex tubifex	-	-	-	-
Potamothrix hammoniensis	-	-	-	-
Rhyacodrilus coccineus	-	-	-	-

Discussion

It is obvious that taxonomic data processing affects cluster results. To improve cluster results, it would be valuable to standardise sampling and sorting methods, sample in the same season, use the same identification keys, and always try to identify to species level. The data set is then as consistent as possible.

If a data set is not consistent, the data should be processed taxonomically before analysis. The two data processing methods that were used in this study showed different results. There was no best method, both had advantages and disadvantages.

Aggregation of species resulted in fewer clusters than omitting the genus or family. This was caused by a higher number of taxa in the 'species' data set, which resulted in a higher differentiation. The combination of species inhabiting a specific

environment comprises a cluster. The advantage of a 'species' data set is highest if species within a group differ ecologically and are abundant.

On the other hand, if within a genus or family no species are identified or the occurrence and abundance of the identified species are low, clustering is not improved if species are retained in the data set. Samples resemble each other because of the absence or low number of species within a certain taxonomic group. In that case it is better to aggregate the species to a higher taxonomic level. These levels are often more abundant. The occurrence together with abundance of genera/families then determines the distribution of the samples over the clusters.

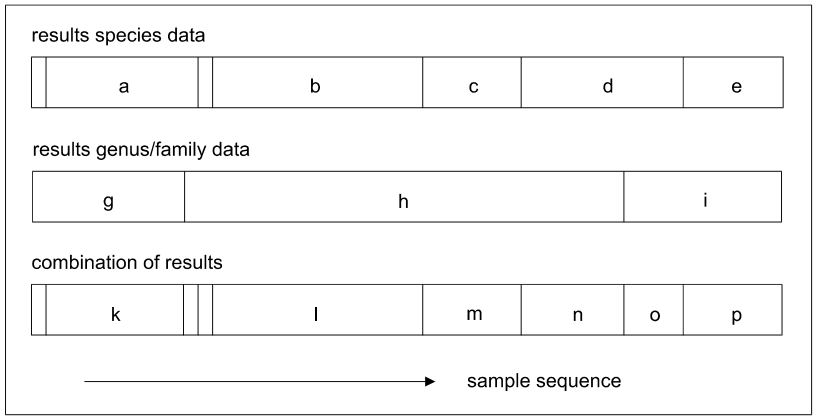


Fig. 2. Distribution of samples over the clusters resulting from clustering with 'species' and 'genus/family' data (treshold value was 0.15). Each cluster is indicated by a letter.

Both processing methods can be used within one data set. The choice of the most suited processing method should be made per taxonomic group, based on the combination of occurrence, abundance and ecological relevance of the species within a taxonomic group. If species occur in many samples, numbers of specimens are reasonable, and species differ ecologically, they should be kept in the data set. Otherwise, aggregation is preferable. If occurrence and abundance of species and genus/family of a taxonomic group are comparable, both methods should be used, thus the data should be analysed twice. The cluster results can then be combined as is demonstrated in Fig. 2. The combination results in more clusters which can be aggregated by investigation of the species composition of the samples with the help of a cluster table.

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3 Rare and common macroinvertebrates: definition of distribution classes and their boundaries

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Abstract

Rarity of macroinvertebrates can be used in assessing the ecological quality or conservation value of freshwaters. To select target species for nature conservation and to compare rarity or commonness between regions a classification of species distributions is needed. A distribution classification for Dutch macroinvertebrate taxa is presented. Frequencies of occurrence in surface waters of aquatic macroinvertebrate taxa were extracted from databases of water district managers and included data from 7608 sites from 1980 to 1998. Six classes were defined: very rare, rare, uncommon, common, very common, and abundant. The boundaries for these classes were established using the number of occurrences of the taxa and the expert opinion of a committee of water district managers and taxonomic experts. A distribution class was assigned to each of 1544 taxa, including 1514 species and 30 taxa on higher taxonomic level (groups, aggregates, genera). There were still many taxa for which the distribution was unknown due to their small size, their special habitat requirements, or identification difficulties due to immature animals and incomplete taxonomic information. The number of rare species differed between the 24 water management districts and depended on characteristics of the area, sampling strategy, and sampling intensity. The percentage of rare species differed between taxonomic groups. The definition of distribution classes and their boundaries can be applied at different scales, (e.g., water management district or national scale), which makes results comparable between scales and provides a broad application in bioassessment and conservation.

Introduction

Identification and protection of rare and endangered species is a central issue for conserving biodiversity (Boon 2000). Rare species may also be critical for bioassessment (Cao *et al.* 2001). In 1966 the International Union for Conservation of Nature and Natural Resources (IUCN) initiated the development of Red Data Books of threatened species (Simon *et al.* 1966a, b). First, species conservation mainly focused on vertebrates (Hafernik 1992), because they are better known and more charismatic than invertebrates. Over the past five to ten years, national and international conventions and statutes addressed the conservation needs of invertebrates, e.g., the Habitats Directive (European Centre for Nature Conservation 1992) has included 59 invertebrates out of 625 species for which special areas of conservation should be designated (Boon 2000). Until now, few Red Data Books for invertebrate groups have been developed, e.g., for Odonata in Belgium (De Knijf & Anselin 1996). Red Data Books have an official political status and can be used for: (1) revealing the threatened status of macroinvertebrate species to the public, research institutions, and national and regional governments, (2) protection, restoration, and management of the environment in which threatened species occur, (3) prioritising areas for protection against disturbing activities, (4) formulating research programs concerning populations of rare species (Maes *et al.* 1995). In the Netherlands, Red Data Books are used for the selection of target species. Target species are protected by law and thereby their habitat is protected. Although macroinvertebrates are a major group in fresh waters, they have yet to be included in the Red Data Books or in lists of target species. Recently, this was done for Tricladida, Trichoptera, Ephemeroptera, and Plecoptera in the Netherlands (Verdonschot *et al.* 2003). Besides a declining trend in distribution range, rarity is one of the major criteria for listing a species in the Red Data Books. For selection as a target species in nature policy, the species must also be of international importance.

In bioassessment, the distribution of species and especially rare species is important as well. Rare species are one of the most reliable indicators of degradation for many aquatic groups (e.g., Lyons *et al.* 1995, Cao *et al.* 1998). Common species are often widely distributed (Gaston 1994) and associated with unstable and disturbed habitats more frequently than rare species (e.g., Thomas 1991, Rakocinski *et al.* 1997). Including rare species might result in a more accurate and sensitive bioassessment (Cao *et al.* 2001). Therefore, the presence of rare species should be a variable in monitoring restoration projects and assessing ecological quality. An example is the System for Evaluating Rivers for Conservation (SERCON), in which rarity is one of the attributes (Boon *et al.* 1997).

There are diverse causes for rarity, e.g., a low dispersion and colonisation ability, physical and chemical environmental constraints, a relation to a specific rare or fragmented habitat, or biotic interactions (Gaston 1994, Lawton 1999).

Rarity can be defined either by the species' abundance or by the species' distribution range (Gaston 1994). Often, rare species are referred to (1) species occurring at many sites but in low densities or (2) species occurring at a few sites in low or high numbers (Gauch 1982). The choice of using either abundance or distribution range for the definition of rarity depends on the cause of rarity that one would like to consider. Nature and biodiversity conservation often focus on species that are rare because they inhabit special habitats, or because of environmental degradation or over-harvesting. Both groups of rare species are threatened. In bioassessment, those rare species that indicate natural environmental conditions without human impact are useful. Rarity caused by pollution or habitat degradation is related to environmental or habitat constraints and therefore characterised by a small distribution range rather than low abundance. At sites with suitable environmental conditions, the abundances of these species can be high, although they only occur at few sites. Because these species should not be excluded from the group of rare species to define rarity of species for use in bioassessment or nature conservation the focus should be on distribution range only.

Although rare species need protection and could be a suitable component of bioassessment procedures, Dutch water district managers do not often focus on rare species, for two main reasons. First, the chance that rare species are collected during standard sampling is less than for more common species. Second, there is no knowledge about which species are rare and which are not. Knowledge is needed about species distributions, at a regional and a national scale. Water district managers have knowledge about species in their own region but data about species distributions are not yet combined and analysed on national scale. Therefore, it is necessary to collect all available data from regional water managers and compare species distributions on a national scale. Criteria for the definitions and classification of rarity of macroinvertebrates are needed. To compare the rarity of species, criteria need to be applicable on regional and national scales. Rarity can be regarded as a continuous variable, but it is more practical for managers to use categories (Gaston 1994). Therefore, distribution classes have to be defined and class boundaries should be set.
The objectives of this study were:

- To define distribution classes and their boundaries for aquatic macroinvertebrates in the Netherlands;
- To draw up a list of macroinvertebrates and their distribution classes on a national scale;
- To compare the number of rare species among water management districts and among taxonomic groups.

Methods

Data collection

The Netherlands are divided in about twenty water management districts, based on province boundaries, water shed boundaries, or polder areas (Fig. 1, Table 1). Water district managers in the Netherlands conduct routine sampling of macroinvertebrates in their surface waters. Each water district manager has a monitoring network in which a high number and variety of surface waters is included. Data are collected in databases maintained by these water district managers. These data include macroinvertebrate samples from a great variety of water types all over the country. Samples are taken in different seasons. Most water district managers sample their waters twice a year, mostly in spring and autumn. Water district managers use a standard sampling procedure (Verdonschot 1990). First a representative part of the surface water is chosen. Subsequently the distribution of habitats for that part is determined. Macroinvertebrate samples from each major habitat are taken in proportion to the present surface area of the habitat. Macroinvertebrates are sampled with a pond dip net (mesh-size 500 μm) of 30 cm width over a total length (including all sampled habitats) of 5 m. The habitat samples are put together in one bucket. Macroinvertebrates are sorted in the laboratory and identified by the water district managers.

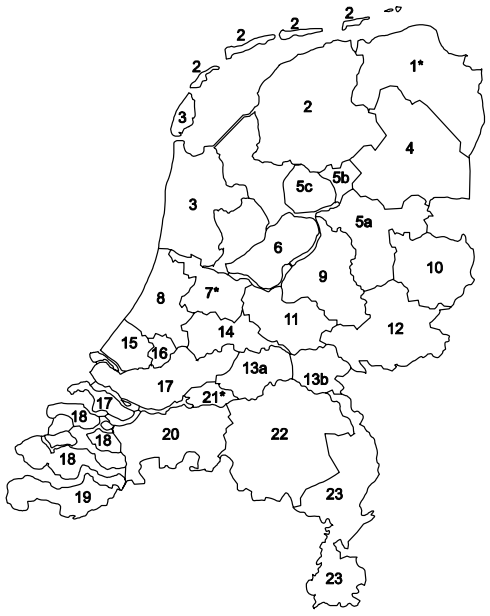

Fig. 1. Map of the Netherlands with water management districts. Numbers indicated on the map are included in Table 1 (* no data available).

Table 1. Original number of taxa, number of taxa after taxonomic adjustment and number of sites for each water management district ('number on map' refers to Fig. 1).

water management district	number on map	land area (km^2)	original number of taxa	number of taxa after adjustment	number of sites
Groningen	1	2377	-	-	-
Friesland	2	3526	653	363	136
Uitwaterende Sluizen	3	1956	777	519	937
Drenthe	4	2685	783	620	164
Salland	5a	1691	1165	675	454
Wieden & Weerribben	5b	288	862	511	188
Noordoostpolder	5c	480	385	224	56
Flevoland	6	999	484	287	32
Amstel, Gooi & Vecht	7	769	-	-	-
Rijnland	8	1070	809	427	201
Veluwe	9	1332	1296	750	394
Twente	10	1334	1690	943	510
Vallei & Eem	11	1098	1227	685	236
Achterhoek	12	1866	1105	733	266
Betuwe	13a	762	963	470	186
Rijk van Nijmegen	13b	571	741	574	32
Stichtse Rijnlanden	14	799	851	466	136
Delfland	15	379	380	315	227
Schieland	16	192	509	346	102
Hollandse Eilanden en Waarden	17	1676	917	576	417
Zeeuwse Eilanden	18	829	459	275	386
Zeeuws Vlaanderen	19	744	451	272	158
West-Brabant	20	1675	861	460	97
Alm en Biesbosch	21	238	-	-	-
Oost-Brabant	22	3113	1268	771	487
Limburg	23	2208	1460	847	639
Noord-Holland (provincial database)	3, 7, 8	3795	1119	804	1167

The data used for our analyses consisted of a taxa list with the number of sites at which each taxon was found in each of 24 water management districts over the period 1980-1998 (based on all samples of macroinvertebrates in their databases from this period). The total number of sampled sites per water management district was counted (Table 1). Overall, 7608 sites were sampled. The data included different water types (e.g., rivers, streams, channels, ponds, moorland pools) but the number of sites was not equally distributed over these water types, with channels and streams being overrepresented, because of their relatively high occurrence. Taxa abundances were not included, because these were not comparable between data sets and because we preferred to define distribution classes based on number of occurrences as explained

in the introduction. Usually, the number of individuals is about 1500 in a macroinvertebrate sample of a water district manager. The data were combined in a table that included the taxon names, the number of sites for each taxon per water management district, the name of the district, the total number of sites for each district, and the percentage of sites per district where each taxon was observed.

Data preparation

Before analyses were undertaken some adjustments were made to make the taxon lists comparable between regions:

- Non-macroinvertebrate taxa and families that were only occasionally found were excluded: fishes, amphibians, Bryozoa, Cnidaria, Polychaeta, Spongillidae;
- Marine taxa, which only occurred in a few salty creeks were excluded;
- Terrestrial species were excluded;
- Exuviae were excluded, because they were not representatively sampled and because they sometimes do not originate from the site at which they are found;
- Similar taxa but in a different life stage, e.g., larvae, juveniles, adults, pupae, were considered as one taxon;
- Old taxon names (synonyms) were replaced by currently used names according to the TCN-list (Taxon Coding Netherlands, VAN DEN HOEK *et al.* 2001);
- Species unknown from the Netherlands were removed (they are assumed to be unreliable identifications);
- All taxonomic units higher than species level, i.e. genus, were excluded from the data if taxa within the respective group on a lower level, i.e. species, were present.

After preparing the data set, 1589 taxa were left of which 6 were families, 91 genera, 22 species groups or aggregates, and 1470 species. This taxa list was used to calculate:

- The number of districts in which each taxon occurred;
- The number and percentage of sites at which each taxon occurred in each water management district
- The total number and percentage of sites at which each taxon occurred at the national scale.

Establishment of the distribution classes

A committee including researchers, taxonomic experts and experienced water district managers established 6 distribution classes by setting class boundaries at the national level (Table 2). Therefore a complete taxonlist was made, ordered by the number of occurrences for each taxon in the Netherlands, from the taxon with the lowest number of occurrences to the taxon with the highest number of occurrences. Each individual on the committee had to divide this list into 6 groups by drawing a line between taxa belonging to two successive distribution classes. After that, the

boundaries drawn by each individual were compared and discussed before the final decision was made (Table 2). Criteria used for the final decision were the average of the boundaries chosen by the committee members and the knowledge about habitat and distribution species close to the boundaries. In this way, the 1589 taxa were assigned to one of the six distribution classes, based on their number of occurrences (calculated distribution classes).

Table 2. Distribution classes and their boundaries (numbers of sites), ranges for lower and upper boundaries as proposed by the committee of experts (numbers of sites), and the final ranges expressed in numbers and percentages of sites.

distribution class	range for lower boundary as proposed by the committee (numbers of sites)	range for upper boundary as proposed by the committee (numbers of sites)	final range (numbers of sites)	final range (% of sites)
very rare	-	10-14	0-12	0-0.15
rare	11-15	35-50	13-43	>0.15-0.5
uncommon	36-51	80-174	44-128	>0.5-1.5
common	81-175	287-400	129-342	>1.5-4
very common	288-401	978-1076	343-1032	>4-12
abundant	978-1077	-	>1032	>12

Correction assignment of taxa to distribution classes

The list with 1589 taxa assigned to one of the six distribution classes, using the numbers of occurrences (calculated distribution classes), was distributed among taxonomists with expertise on Odonata, Oligochaeta, Chironomidae, Trichoptera, Hydracarina, and Coleoptera. Using their knowledge and the results of other studies in the Netherlands (Mol 1984, Gittenberger *et al.* 1998, Drost *et al.* 1992, Geijskes & Van Tol 1983, Bos & Wasscher 1997, Smit & Van der Hammen 2000) the distribution classes were adjusted for some taxa (Table 3: assigned distribution class). Two types of adjustment were necessary. The first type of adjustment concerned 284 taxa that were considered by experts to be more common than indicated by the occurrence data. These often occurred in habitats that were sampled only occasionally by a water district manager. It was felt that taxonomic experts were more aware of the specific distribution of these taxa. The second type of adjustment concerned taxa that were thought to be rarer than indicated by the data (42 taxa). These taxa may have been unreliably identified, due to barely distinguishable features of small animals or because of recent changes in taxonomy. For each taxon whose distribution class was altered the reason for the adjustment was given in separate columns of the list. Taxa whose distributions are unknown, because only some water district managers identified them to species level and their distributions were not known by the expert panel, were not

given a distribution class (253 taxa) (Table 3). These taxa were mainly Diptera whose taxonomy is still not well developed in the Netherlands.

Table 3. Number of taxa per distribution class based on number of occurrences in the data set (calculated distribution classes), versus distribution classes assigned by the expert committee (assigned distribution classes).

	assigned distribution class (number of taxa)								total per calculated distribution class
calculated distribution class	abun-dant	very common	com-mon	uncom-mon	rare	very rare	extinct	no class	
abundant	100	2						1	103 (6%)
very common	10	121	3					5	139 (9%)
common	7	18	137	4	2			10	178 (11%)
uncommon	2	16	47	146	11	3		12	237 (15%)
rare	3	9	10	46	160	16		31	275 (17%)
very rare	5	8	20	25	58	346	1	194	657 (41%)
added from national list		1	3	9	23	107	65		208
total per assigned distribution class	127 (8%)	175 (11%)	220 (14%)	230 (15%)	254 (16%)	472 (31%)	66 (4%)	253	1797

Additional species

To complete the list, 208 species that are known from the Netherlands but were not found by the water district managers were added. Most were known to be very rare (evaluated by the taxonomic experts) or probably extinct from the Netherlands. Species about whose distribution nothing was known, were not included in the list. In total, a distribution class was assigned to 1544 taxa, including 1514 species.

Regional differences

For each water management district the total number of species and the number of species in each distribution class were calculated. For the species in each distribution class the mean number and standard deviation of water management districts was calculated. The same was done for the mean percentages of sites at which species within a distribution class occurred.

Results

The aquatic macroinvertebrates' distributions list

The complete list with all taxa and their distribution classes can be found on the internet (www.alterra.wur.nl). An example of the complete information per taxon is given for the Tricladida (Table 4).

Table 4. The Tricladida as an example extracted from the Dutch rare aquatic macro-invertebrate list.

taxon name	number of districts	number of sites	% of sites	calculated distribution class (based on frequency of occurrence)	assigned distribution class (adjusted by taxonomic experts)	remark
Dugesia gonocephala (Dugès)	2	106	1.2	uncommon	rare	only in specific environments, cold stenotherm species, only in 2 regions
Dugesia lugubris (Schmidt)	24	1358	15.8	abundant	very common	
Dugesia polychroa O.Schmidt	21	907	10.5	common	common	
Dugesia tigrina Girard	23	483	5.6	common	common	
Planaria torva (Müller)	14	117	1.4	uncommon	uncommon	
Polycelis felina (Dalyell)	5	65	0.8	uncommon	uncommon	
Polycelis nigra (Müller)	21	701	8.1	common	common	
Polycelis tenuis Ijima	25	1470	17.1	very common	abundant	
Crenobia alpina (Dana)	4	12	0.1	very rare	very rare	
Dendrocoelum lacteum (Müller)	25	938	10.9	common	common	
Bdellocephala punctata (Pallas)	8	17	0.2	rare	rare	
Phagocata vittata (Dugès)				added	very rare	ground water species

Number of taxa in each distribution class

Fig. 2 shows that there are many taxa that occur on only one or a few sites. The number of taxa that occurs at a high number of sites is low.

The classification of taxa into 6 distribution classes resulted in a distribution from few abundant taxa (8%) towards many very rare taxa (31%) (Table 3). Very rare taxa were found at 12 sites or fewer over a period of 18 years.

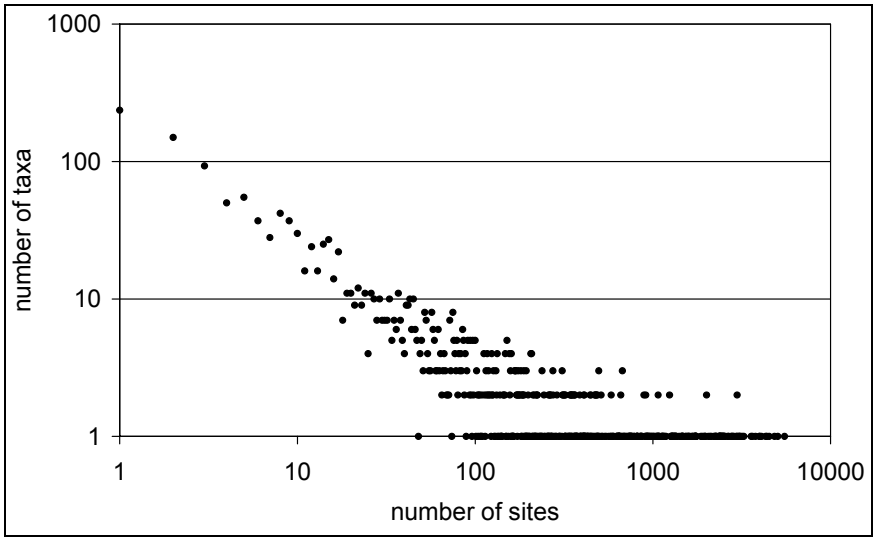

Fig. 2. The number of taxa that occur at a certain number of sites.

Distribution of rare species over the water management districts

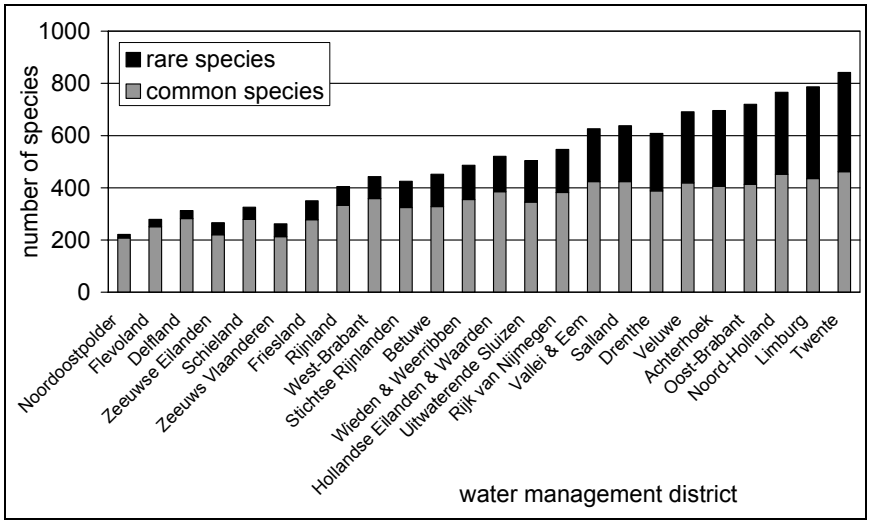

Fig. 3. Number of rare species (very rare, rare and uncommon) and number of common species (common, very common and abundant) for each water management district. Taxa on higher level, i.e., genera and families are not included.

The numbers of rare species differed between water management areas (Fig. 3). In some districts, e.g., the Noordoostpolder, only few rare species were collected, whereas in other districts, such as Limburg and Twente, almost 250 rare species were

found. Districts in the southern and eastern parts of the country had the highest numbers of rare species. Districts in the middle of the country had low numbers of rare species.

Table 5. Mean number and coefficient of variation of water management districts in which species within a distribution class occur, and mean percentage and coefficient of variation of sites per water management district at which species in each distribution class occur (taxa on higher level, i.e., genera are not included).

distribution class	number of water management districts		% of sites per water management district	
	mean	coefficient of variation	mean	coefficient of variation
abundant taxa	24.0	0.06	26.7	0.76
very common taxa	19.4	0.24	8.5	1.03
common taxa	14.9	0.32	4.1	1.23
uncommon taxa	9.71	0.42	2.2	1.27
rare taxa	5.73	0.50	1.3	1.39
very rare taxa	2.03	0.67	0.6	1.90

The mean number of water management districts in which particular species occur was highest for abundant species and lowest for the very rare ones (Table 5). The variation in the number of water management districts in which a species was found (indicated by the coefficient of variation) was low for the abundant and high for the rarer species. This means all abundant species occurred in a high number of water management districts, while the very rare species showed some more variation in their number of water management districts.

The mean percentage of sites at which particular species occurred within water management districts also decreased towards the rarer species (Table 5). Again, the coefficient of variation was higher for the rarer species, indicating that the percentage of sites at which they occur within a water management district was more variable than for the more abundant species.

Distribution of rare species among taxonomic groups

The percentages of rare species (distribution classes uncommon, rare, and very rare) within each taxonomic group varied from 29% for Gastropoda to 91% for Plecoptera. The groups with the highest percentages of classified rare species were Plecoptera, Trichoptera, Odonata, Ephemeroptera, Chironomidae, Coleoptera, and Hydracarina (Table 6). Ephemeroptera, Plecoptera, Hydracarina and Trichoptera included the highest number of extinct species.

Table 6. Numbers of species per distribution class, total numbers of species, and percentage of uncommon, rare and very rare species for each taxonomic group (taxa on higher level, i.e., genera are not included).

taxonomic group	abundant	very common	common	uncommon	rare	very rare	extinct	no class	total	% uncommon rare and very rare
Plecoptera		1		1	3	6	18		29	91
Ephemeroptera	3	3	2	4	13	22	15		62	83
Trichoptera	6	22	14	25	29	70	12	1	179	75
Hydracarina	10	28	25	38	34	69	17		221	69
Diptera: Chironomidae	25	31	60	63	56	141		8	384	69
Odonata	1	7	14	8	10	26			66	67
Coleoptera	25	28	41	34	55	92	2	23	300	66
Diptera: non-Chironomidae	1	5	9	7	12	8	1	196	239	64
Crustacea	4	8	4	6	6	7		3	38	54
Oligochaeta	5	11	22	17	18	10		14	97	54
Tricladida	2	4		2	2	2			12	50
Heteroptera	11	12	13	8	11	8			63	43
Hirudinea	8	1	1	3		4			17	41
Lepidoptera	1	1	1	1	1				5	40
Bivalvia	4	8	6	5		5		1	29	36
Gastropoda	19	4	6	7	3	2	1	4	46	29
total	125	174	218	229	253	472	66	250	1787	65

Discussion

The definition of distribution classes

Distribution classes can be defined based on absolute or relative abundance or occurrence. In this study relative numbers of occurrences were chosen. This is preferable if a general definition is needed and rarity of species is to be compared on different scales or between different geographical areas on the same scale (Gaston 1994). Six distribution classes were used, the same number used in a national study on the distribution of Trichoptera (Higler 1995).

There are two options for defining distribution classes. The first is using the number or percentage of sites. For example, Collins & Glenn (1990) used number of watersheds for plant species and Tonn *et al.* (1990) used the number of lakes for fishes. The second option is to use surface area, e.g., Verkaar (1990) used 25 km^2 grid squares for occurrence of plants and Longton (1992) used 10 km squares for mosses. In this study the number of sites was used. For macroinvertebrates, which are bound to more or less isolated surface waters, this is probably the better criterion. The two measures can result in very different classifications. Presence of a species within one

surface quadrant can range from occurring in many water bodies within the quadrant to only one. Species that are observed at many sites can be restricted to a small geographic area or be widely distributed over the country. If a species occurs at only a few sites but the sites are spread over the whole country the species would be considered as rare if the number of sites was used as a criterion but the species would be considered as common or widespread if the number of surface quadrants is used. To compensate for the lack of geographic distribution data (co-ordinates were not available) in our study, the number of districts in which a taxon occurred was included in the verification procedure that was carried out by the experts after the initial assignment of distribution classes. Taxa that were restricted to a very small area (only one district) were assigned to a lower distribution class than was originally calculated from the occurrence data. Probably, the combination of both the number of sites and geographical distribution would give the most optimal classification.

In fact, the percentage of occurrences of species is a continuous variable. Still, categories are used. The major problem with this approach is how to determine the boundaries for each of the distribution classes. The choice of these boundaries is arbitrary (Gaston 1994). In our study, a committee of water district managers and taxonomic experts defined the boundaries of the distribution classes. In general, the choices of the class boundaries made by the individuals in this committee were quite similar (Table 2). However, during checking the distribution class of the individual taxa there were sometimes different opinions about the distributions because of regional differences.

Over- and underestimation of rarity

The assignment of distribution classes to taxa based on frequency of occurrence, as done in this study, could have resulted in an under- or over-estimation of rarity. There are five factors that could lead to errors in assigning distribution classes to taxa. First, there probably was a sampling bias to high abundant taxa. Wright *et al.* (1992) emphasised that whereas a high frequency of occurrence may confirm that a taxon is common, a low frequency cannot be assumed to indicate a rare taxon. This is partly due to the relationship between the abundance of the taxon and the chance that it is collected during sampling. Taxa that are not abundant have a smaller chance to be collected during sampling and therefore their distribution range could be underestimated. Although the samples were large, the determination of the distribution class might be more reliable for taxa with a high abundance than for taxa with a low abundance.

Second, differences in sampling intensity between water district managers resulted in a skewed distribution of the number of sampling sites over the country (Table 1). Because we only retrieved the numbers of occurrences of the taxa, we could not make a further selection in the data to obtain a balanced sampling design. This

could have affect the evaluation of the distribution class of a taxon, especially if the representation of water types differs between the water management districts, which is the case in the Netherlands and probably also in other regions. Stevens (1994) suggested that it is better to relate the number of samples to the density of water body types in the area rather than take the same number of samples in each area.

Third, rare habitats, such as wetlands and water bodies in nature reserves, owned by nature conservation agencies, were poorly represented because these are not within the responsibility of the water district managers. Streams and channels (used for drainage of agricultural areas) were most often sampled.

Fourth, the distribution of a number of taxa is unknown because they were infrequently identified to a low, preferable species level. It mainly concerned Oligochaeta, Hydracarina, Pisidiinae, and Diptera. For a number of taxa from these groups the number of occurrences was not reliable. Taxonomic experts could improve the distribution classification for a part of these taxa, but there were still many taxa left without a distribution class, mainly Diptera.

Fifth, the rarity of small species might be overestimated because the mesh size used was 500 µm. The percentage of small animals might be smaller in the sample than in the water body sampled. This is only critical for species that remain small during their whole life cycle. For species which are small only during the first larval instars this is no problem, because samples from different seasons were included.

To improve the macroinvertebrate distribution list, (1) more samples from rare habitats should be added, (2) an even distribution of the sampled sites over the country should be used, e.g., a random sampling design depending on the distribution of water types and their densities in an area (Stevens, 1994), (3) co-ordinates of the sites should be included to know the exact geographical range of a species, (4) identification should be completed at species level, and (5) the distribution of small species should be further studied using a smaller mesh size. This asks for more research in rare habitats, standardisation of sampling, sorting and identification methods by water district managers and further development of taxonomy and identification keys on Dutch macroinvertebrates.

Scale of the rarity list and regional differences

Rarity is often used at different scales, e.g., at regional and national scale in the water management program EKO (unpublished data) and at European (EC Habitats Directive), and national scale in SERCON (Boon 2000), a system for evaluation of river conservation value. In our study one national list with taxa distribution classes was constructed but it is of importance to complete regional lists as well, because of differences of distributions between districts. Therefore, the same class boundaries can be used, based on the percentages of occurrences within the region. After

calculating the regional distribution classes experts from the region have to check the classes for all taxa to improve the regional lists.

The national list can be used for national studies on nature and water management. Water district managers can also use this list to determine which of the taxa in their district are rare on a national scale. With the help of regional rarity lists, water district managers can determine the specific distribution class of taxa for their own district. A taxon that only occurs frequently in one district can be rare at the national scale (e.g., taxa living in the fast flowing hill-streams in the far southern part of the Netherlands). Conversely, a taxon can be common in the Netherlands but rare in a certain district. This for example, can occur if a region does not include suitable habitat for the species. Our study indicated that both situations, rare on the national scale and abundant in a certain region, and abundant on the national scale but rare in a region, occurred frequently. Water managers could use both scales in combination to obtain a better insight of the importance of the taxa found in their district and the need for protection of these taxa and their habitat.

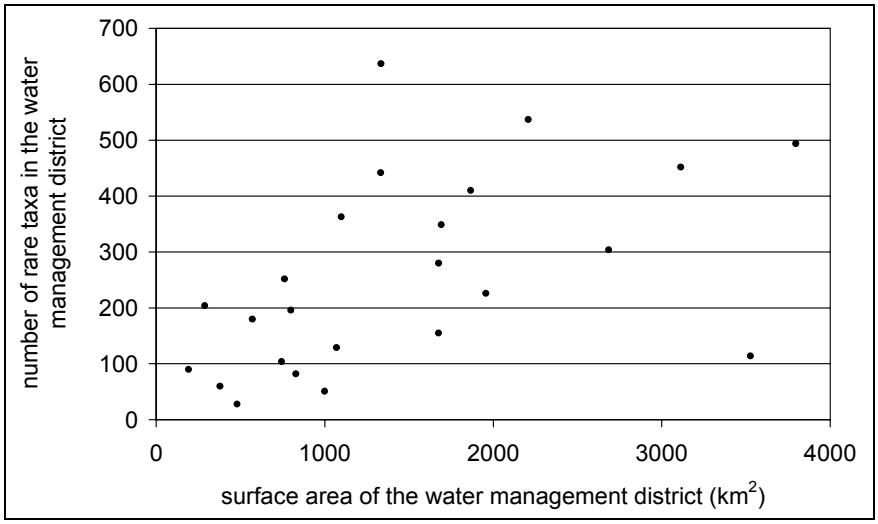

Fig. 4. The relation between the surface area of the water management districts and the number of rare species observed in the districts.

While developing the national rarity list, it became apparent that there were large differences between the numbers of rare species in the water management districts (Fig. 3, Table 1). This could partly be explained by landscape features of the districts, especially:

- Surface area of the district. Fig. 4 shows that especially small water management districts have low numbers of rare species. However, most of the small water

management districts are located in the western part of the country and have no running waters, thus also a lower diversity in water types. Larger areas can either have a low or a high number of rare species;

- Diversity of water types in the area, e.g., Limburg is an area with running and stagnant waters, with lowland and hills and has a high number of rare species;
- Geological age of the area, i.e., the areas Flevoland and Noordoostpoder are polder areas, which contain a low number of species and few rare species, probably because a number of species did not yet succeed in colonising these areas;
- The number of pristine waters in the area, which, for example, is large in Twente, an area with a high number of species and a high number of rare species.

However, these characteristics could not in all cases explain the observed differences. In the district of Noord-Holland for example, the number of rare taxa was high, although this district is comparable with the other districts close to the North Sea. Sampling and processing the sample can influence the number of taxa found, e.g., the total area sampled (Marchant 1990, Rodriguez & Wright 1991, Vinson & Hawkins 1996), the habitats included in the sample, the sampling device (Cheal *et al.* 1993, Goretti & Giovanni 1998, Humphries *et al.* 1998), the diversity of the sampled water bodies, the temporal distribution of the samples (Barbour *et al.* 1992), the sorting and sieving effort (Dukerschein *et al.* 1996, Vinson & Hawkins 1996), and the identification level (Lenat & Resh 2001). Although the sampling protocol used was the same, sampling, sorting, and identification intensity might have differed between water managers.

To compare numbers of rare taxa for use in bioassessment and monitoring between districts, it is necessary that water managers further standardise their sampling and sorting methods and identify to species level if possible.

Rarity related to taxonomic groups

We found differences in numbers of rare species among taxonomic groups. Ephemeroptera, Plecoptera, Odonata, Hydracarina, and Trichoptera had many species that were rare or even very rare (Table 6). Many species in these groups are bound to special habitats or are susceptible to pollution or disturbance. The latter reason is why the number of Ephemeroptera, Plecoptera, and Trichoptera taxa is often used as an indicator of ecological quality, especially in running waters (Lenat 1988). Deterioration of habitats or water quality could be a cause of the decline in number of these species. Bivalvia and Gastropoda included relatively few rare species. Many species of these groups occur in standing waters, such as channels of which many are available in the Netherlands and included in the data.

However, the survey methodology was not standardised for each taxonomic group separately. This could have led to bias towards those groups that are less motile

and tend to remain fixed in a given location. Also the definition of a site could be different for each taxonomic group due to differences in occupied area by one population and its motility. Class boundaries could be set differently for each taxonomic group, but this would make the application in practise more complex. Besides, within taxonomic groups there could also be differences in motility and occupied area by one population.

Species within some taxonomic groups, including high numbers of rare species, could in fact be more common, because of poor taxonomic knowledge. Diptera and Chironomidae were often not identified to species level, which resulted in low numbers of occurrence for some species, which actually are more widespread.

Towards a national Red List for aquatic macroinvertebrates

To give rare aquatic macroinvertebrate species official status, it is important to establish a Red Data Book. The occurrence of threatened species should oblige water district managers to protect the habitat of those species. However, there are two criteria for listing species in the Red Data Books: (1) rarity and (2) a negative trend in the species' distribution. Species that are selected as target species in the Dutch nature policy should also be of international importance (small distribution elsewhere). This research resulted in assignment of distribution classes but the trends in distribution are still unknown for most of the species. The international importance can be determined by analysing data from other European countries to establish the species' distribution in other countries. To further develop the rarity list into a Red Data Book, it is therefore necessary to monitor the trend, both short-term and long-term, in the number of occurrences of taxa over time. This is possible if comparable data for all taxa are to be collected again periodically. The new results can be compared with the data that were used in this study (1980-1998). The decrease or increase of taxa can then be defined by using percentages of change. By calculating the occurrence of taxa every five years, trends in distribution ranges of taxa can be followed closely, and threatened taxa can be detected early. This provides the opportunity to take protection or restoration measures in time to protect them.

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4 The effect of excluding taxa with low abundances or taxa with small distribution ranges on ecological assessment

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Abstract

The present study aims to investigate whether taxa with a small distribution range or taxa with low abundances indicate specific habitats or a high ecological quality and what the effect is if these taxa are excluded from ecological assessment.

We compared autecological features between stream dwelling taxa with a mean abundance > 5 individuals per sample and a mean abundance ≤ 5 individuals per sample as well as between taxa with a small distribution range and taxa with a large distribution range. The number of rare taxa (either with a small distribution range or with low abundances) in a sample was related to the ecological quality classes. To test the effect of exclusion of rare taxa we constructed 8 data sets all including 142 samples of Dutch lowland streams. From each data set we stepwise excluded taxa that had low abundances or taxa that were known to be restricted in their distribution range. With help of the AQEM assessment software we calculated the final ecological quality classes and the metrics that were included in the multimetric for the original data and the 8 selected data sets.

Autecological features of the taxa within the different selections showed that taxa with small distribution ranges were often running water taxa, living on stones and gravel and indicating oligosaprobic water conditions in contrast to taxa that had a large distribution range. There were only small differences between taxa with low and high abundances. However, current velocity preference was lower for taxa with abundance ≤ 5 individuals per sample, saprobic values were higher and scores for typical stream habitats, such as lithal, psammal and akal were lower compared to high abundant taxa.

If taxa with low abundances were excluded a higher ecological quality class was achieved in most cases, while excluding taxa with a small distribution range resulted in lower ecological quality classes.

In conclusion, excluding taxa with a small distribution range led to worse ecological quality classes because these taxa have special autecological features that

often indicate natural streams. On the other hand, excluding taxa with low abundances resulted in higher ecological quality classes because these taxa indicate more disturbed situations and because the number of taxa per sample was strongly reduced.

Although the documentation of rare taxa (either with low abundances or with small distribution ranges) is often time and cost-intensive regarding field work, laboratory work, data processing, and analyses, the indicative power of these taxa for natural circumstances is essential and therefore rare taxa should be included in ecological assessment studies.

Introduction

In population and community ecology rare species are regarded as those species that have a low abundance and/or a small distribution range (Gaston, 1994). Both, abundance and distribution range criteria are also used to classify rarity of macroinvertebrates in streams and rivers. However, it is the question which type of rarity is of importance in ecological assessment.

Species with low abundances and large distribution ranges are probably not very indicative for specific environmental conditions. Environmental factors could cause low abundances of a species. For example, if species live under sub-optimal conditions in the outer range of their distribution range. Often, species that always occur in low numbers have biological characteristics that prevent a fast population growth, e.g., a long life cycle, few offspring, or dependence on a host species. Also species with different feeding types seem to have different abundances in a community. For example, predators seem to be rarer than prey species (Spencer, 2000).

Species may be limited to a small range of streams because they are geographically restricted (narrowly endemic species) or require specific habitat conditions that are rare (Gaston, 1994; Lawton, 1999). Species with limited distribution ranges are often related to specific environmental factors. However, biological factors might be important as well, e.g., a low dispersion and colonisation capacity or interactions with other species (Gaston, 1994). Species that occur in only few stream types or habitats can have a high or a low abundance at the sites where they occur. The ones with a small distribution range and low abundances are extremely vulnerable.

Rarity of species should always be considered in relation to sampling strategy. Sampling macroinvertebrates always reveals only a part of the present community at a site. Species that occur in low abundances have a lower chance to be sampled and therefore the number of sites at which they are found might be lower than for the species with high abundances that occur at the same sites. Therefore, sampling strategy should be standardised to compare rarity of species. To collect species with

low abundances one should take a sufficient number of replicates. On the other hand, species with a small distribution range occur in only few sections or habitats of a stream, which requires sampling of all available habitats in the stream. In addition, temporal aspects can be important. Some species are only present in the streams during a very short period of the year, although they are not rare. If the sample is taken during a different season these species will not be found.

In aquatic macroinvertebrate communities, species with, low abundances may form the largest component of species richness (Marchant *et al.*, 1997; Cao *et al.*, 1998; Lenat & Resh, 2001). However, rare species (either with low abundances or occurring in only few samples) are often excluded from analyses, because (1) including rare species (either with low abundances or with small distribution ranges) in sampling, sorting, and identification processes is time-consuming and (2) it is believed that rare species in a sample might be present by chance and therefore only add noise to the analysis (Gauch, 1982; Marchant, 2002). Exclusion of taxa can take place (1) during sorting, e.g., by subsampling (taxa with low abundances are excluded), (2) by specific identification procedures in which it is not necessary to identify further if a rare taxon is concerned (taxa with small distribution ranges are excluded), and (3) during data preparation by setting relative abundance or frequency of occurrence criteria (taxa with low abundances or small distribution ranges are excluded, respectively) (Gauch, 1982; Pardo & Armitage, 1997; Hawkins & Vinson, 2000).

Excluding rare species from analyses has been criticised because valuable information can get lost (e.g., Cao *et al.*, 1998, 2001; Karr & Chu, 1997; Lenat & Resh, 2001). Rare species (especially those with small distribution ranges) may be good indicators for ecological quality (e.g., Lyons *et al.*, 1995; Lenat & Resh, 2001). This implies that rare species are important in ecological assessment and exclusion of rare species requires careful consideration (Cao *et al.*, 2001).

The research project 'The Development and Testing of an Integrated Assessment System For the Ecological Quality of Streams and Rivers Throughout Europe using Benthic Macroinvertebrates' (AQEM) aimed to develop an ecological assessment system based on deviations in ecological quality which are established as the difference between observed conditions and the reference condition (condition with no or minimal anthropogenic stress) (Hering *et al.*, 2004). Therefore, a multimetric (which consists of several metrics or indices, based on indicative values of species, e.g., for organic pollution or functional features) was constructed for each stream type. The complete macroinvertebrate community was included in data processing and the development of the assessment system. However, for potential users of the assessment system it would be more cost-effective if rare species could be excluded from data-processing. However, up to now the effect of excluding rare species either on abundance or on distribution criteria on assessing the ecological quality of a stream is subject of discussion. In this study, we defined two types of rare species: (1) species with a low abundance in a sample and (2) species with small

distribution ranges (occurring at a limited number of sites). We hypothesise that species with low abundances are not indicative for the ecological quality or specific habitats and that species with small distribution ranges are indicative for a high ecological quality or indicate specific habitats. Therefore, we hypothesise that excluding species with low abundances has no effect on ecological assessment but that excluding species with small distribution ranges influences the result of ecological assessment.

Materials and Methods

Data collection

In the AQEM project an assessment system was developed to assess the ecological quality of streams in Europe. As mandated by the European Water Framework Directive (WFD) assessment of the ecological quality should be based on the difference between observed conditions and the reference conditions (European Commission, 2000). Following the WFD criteria, the AQEM project used a typology-based approach for the development of the assessment system.

Consequently, a number of factors were used to partition the natural variability expected to occur at a stream site; streams were classified by ecoregion, altitude, and size of the catchment (Verdonschot & Nijboer, 2004). A human-generated disturbance gradient (e.g. organic pollution) of sites was selected and sampled for each stream type. A minimum of 11 stream sites was chosen for each stream type, consisting of 3 sites of high ecological status (reference conditions, class 5), 3 sites of good ecological status (class 4), 3 sites of moderate ecological status (class 3) and 1 site each of poor (class 2) and bad ecological status (class 1) (European Commission, 2000). The final typology scheme consisted of 30 stream types within the 8 countries (Hering *et al.*, 2004).

In the Netherlands, 142 sites of the stream type 'lowland streams' were selected along gradients of general degradation (the degradation factors 'morphological degradation' and 'organic pollution' could not be separated). In the Netherlands no real reference sites could be found, therefore, it was decided to include the best available sites and classify these sites as good ecological quality (class 4). Expert judgement was used together with a list of criteria for the selection of reference sites, to choose the best available sites. At each site, macroinvertebrates were collected, following the AQEM manual (Hering *et al.*, 2004), which describes sampling site selection, sampling strategy and processing. The individuals were identified to species level if possible (this could not be done in some cases because animals were still in their first instars and limitations in the taxonomic knowledge of some taxa (Schmidt-Kloiber & Nijboer, 2004).

The relation between autecological features and rarity of taxa

To study the relation between taxa within different abundance classes and their indicative value we firstly related different groups of taxa to their autecological features.

Abundance classes

For each taxon the mean abundance was calculated over all samples. We separated the taxa in the data into two abundance classes: (1) taxa with a mean abundance ≤ 5 individuals per sample (low abundance, N=449) and (2) taxa with a mean abundance > 5 individuals per sample (high abundance, N=416).

Distribution classes

Secondly, the taxa were classified according to their distribution range. To classify the taxa into distribution classes, a list with distribution classes for 1624 Dutch macroinvertebrates was used ('Dutch Macroinvertebrate Distribution Classification List', Nijboer & Verdonschot, in prep.). This list was compiled using over 8000 macroinvertebrate samples from all water types in the Netherlands, including streams and rivers, but also standing waters. Each taxon was assigned to a distribution class based on the number of water bodies in which the taxon occurred in that database. The class boundaries used to classify the taxa are given in Table 1. The distribution classes were: 'very rare', 'rare', 'uncommon', 'common', 'very common' and 'abundant'. The list with distribution classes of the taxa was applied to the AQEM database. In total, 865 taxa were collected during the AQEM project in the Dutch lowlands streams. It was not possible to assign a distribution class to 320 taxa (Table 1), 95% of which were genera or families. These higher taxa are not included in the Dutch Macroinvertebrate Distribution Classification List, because species within a genus or family often have different distribution patterns. The other 5% of these taxa were species with an unknown distribution. This is mainly due to taxonomic difficulties of these taxa, which obscure clear ecological preferences and render past records unreliable. We divided the taxa into two distribution categories: (1) taxa with distribution class very rare, rare, or uncommon (taxa with a small distribution range, N=362) and (2) taxa with distribution class common, very common, or abundant (taxa with a large distribution range, N=182).

Table 1. Number of taxa for each distribution class in the Dutch Macroinvertebrate Distribution Classification (DMDC) List and in the AQEM lowland streams.

distribution class	class boundaries	Dutch Macroinvertebrate distribution classification		AQEM lowland streams	
	% of sites in DMDC data	no. taxa	% taxa	no. taxa	% taxa
very rare	0-0.15	480	23	45	5
rare	>0.15-0.5	263	13	56	6
uncommon	>0.5-1.5	242	12	81	9
common	>1.5-4	236	11	122	14
very common	>4-12	192	9	114	13
abundant	>12	145	7	127	15
no distribution class		436	21	320	37

Calculations

Autecological information of taxa was provided by the AQEM taxalist (Hering *et al.*, 2004). In this list European freshwater macroinvertebrates are included with their ecological characteristics. This list was used to calculate for both abundance groups and for both distribution groups:

- The percentage of taxa belonging to each taxonomic group;
- The percentage of taxa for each current velocity preference type: In the taxalist, each taxon is assigned to one of the 7 current velocity preference classes. Taxa with no indication of current velocity preference were excluded from the calculations;
- The mean score for each microhabitat: In the AQEM taxalist the preference for microhabitats is expressed by dividing 10 points to the microhabitats in which the taxon can occur. The most often occupied microhabitat has the highest score. Taxa without any score were not included. Differences in results between taxa with low and high abundances and between taxa with small and large distribution ranges are tested using a t-test;
- The mean score for each saprobic class (in the AQEM taxalist the occurrence under different saprobic conditions is expressed by dividing 10 points to the saprobic classes, in which the taxon can occur. The saprobic class at which the taxon is most often observed gets the highest score. Taxa without any score were not included. Differences in results between taxa with low and high abundances and between taxa with small and large distribution ranges are tested using a t-test.

The number of rare taxa related to ecological quality classes

The indicative value of rare taxa can also be studied using the relation between the number of rare taxa in a sample (based on either abundance or distribution criteria) and the ecological quality class of the site where the sample was taken.

Abundance classes

The taxa were classified according to their abundance. For each taxon the mean abundance was calculated over all samples. Subsequently the taxa were divided into five abundance classes:

1. Taxa with mean abundance ≤ 1 individual per sample (N=126);
2. Taxa with mean abundance >1 and ≤ 2 individuals per sample (N=92);
3. Taxa with mean abundance >2 and ≤ 5 individuals per sample (N=121);
4. Taxa with mean abundance >5 and ≤ 10 individuals per sample (N=102);
5. Taxa with mean abundance > 10 individuals per sample (N=424).

To relate the number of taxa in each abundance class to the ecological quality classes, all samples that were classified within the same ecological quality class (1 to 4) were combined. For each ecological quality class the mean percentage of taxa from each abundance class was calculated.

Distribution classes

To classify the taxa in distribution classes, again the 'Dutch Macroinvertebrate Distribution Classification List' was used. The taxa were assigned to the six classes in this list: very rare, rare, uncommon, common, very common, and abundant. To relate the number of taxa in each distribution class to the ecological quality classes, all samples that were classified within the same ecological quality class were combined. For each ecological quality class the mean percentage of taxa from each distribution class was calculated.

Additionally, the average number of taxa per sample belonging to the distribution classes 'very rare', 'rare', or 'uncommon', was calculated for each ecological quality class. The differences of the average number between the ecological quality classes were statistically tested using a t-Test.

The effect of excluding rare taxa on ecological assessment

Excluding taxa with low abundances

To test the effect of excluding taxa with different abundances on ecological assessment taxa within 4 different abundance ranges were excluded: (1) taxa with abundance of 1individual in the sample, (2) taxa with abundance ≤ 2 individuals in the sample, (3) taxa with abundance ≤ 5 individuals in the sample, and (4) taxa with abundance ≤ 10 individuals in the sample.

Taxa were removed from a sample if the abundance within that particular sample was lower than the criterion. This means a taxon could be included in one sample and excluded in another sample. This resulted in 4 new data sets in which more or less taxa were excluded.

Excluding taxa with small distribution ranges

To test the effect of excluding taxa with different distribution ranges on ecological assessment again the 'Dutch Macroinvertebrate Distribution Classification List' was used to select four groups of taxa to be excluded: (1) very rare taxa, (2) very rare and rare taxa, (3) very rare, rare, and uncommon taxa, (4) very rare, rare, uncommon, and common taxa.

Each taxon within the criterion was removed from all samples in which the taxon occurred, independent of the abundance in a sample. This resulted in 4 new data sets in which more or less taxa were excluded.

Ecological assessment

The AQEM software (Hering *et al.*, 2004) was used for ecological assessment of the 142 lowland streams. In this program a multimetric is included for each stream type. The Dutch multimetric assessment system uses ten metrics (Table 2; Vlek *et al.*, 2004). Each one of these ten metrics is able to differentiate between one particular ecological quality class and the other three quality classes (reference sites, class 5 were not included). If the individual metric scores between the 25th and 75th percentile of its variation for an ecological quality class the sample is assigned to the respective ecological quality class. There will be no result for the metric if the score is below the 25th or above the 75th percentile. The final ecological quality class is calculated by averaging the individual metric results (multimetric). If no results for any of the ten metrics calculated fall within the 25th/75th percentile range no classifications are made for any of the individual metrics and the final result for the ecological quality classification is 'unknown'. Metrics and multimetric results were calculated for the original data set (including all taxa) and for the eight altered data sets. For each sample all metrics were calculated.

Table 2. Metrics included in the multimetric for Dutch lowland streams and the ecological classes they indicate. Criteria for metric selection are explained in Vlek *et al.* (2004) and further explanation of the metrics is given in Hering *et al.* (2004).

metric name	metric description	quality class established
% grazers & scrapers / % gatherers-collectors & filterfeeders	percentage of the abundance of all taxa belonging to grazers or scrapers, divided by the percentage of the abundance of all taxa belonging to gatherers-collectors or filterfeeders	1
% river stretch hypopotamal	percentage of the community (abundance of all taxa) that prefer the hypopotamal zone	4
% microhabitat pelal	percentage of abundance of taxa that have microhabitat preference pelal (mud, grain size < 0.063 mm)	4
% current velocity preference type rheophil	percentage of abundance of taxa that have current preference rheophil, occurring in streams, prefers zones with moderate to high current	4
EPT-taxa / Oligochaeta	number of Ephemeroptera, Plecoptera and Trichoptera taxa divided by the number of Oligochaeta taxa	2
EPT-taxa / % Oligochaeta	number of Ephemeroptera, Plecoptera and Trichoptera taxa divided by the percentage of individuals belonging to Oligochaeta	2
% Gastropoda	percentage of individuals belonging to Gastropoda	1
Gastropoda & EPT-taxa / Oligochaeta	combination of the metrics Gastropoda (number of taxa belonging to Gastropoda) and EPT-taxa / Oligochaeta	3
% river stretch hypopotamal & EPT-taxa / Oligochaeta	combination of the metrics (%)hypopotamal and EPT-taxa / Oligochaeta	3
saprobic index (Zelinka & Marvan, 1961, 1966)	the saprobic index is calculated based on the scores of the taxa in the samples for 5 saprobic classes from xeno-saprobic to poly-saprobic	4

Results

Autecological features of rare taxa

Taxa with low abundances

More than 70% of the taxa with low abundances (≤ 5 individuals per sample) preferred standing or slowly flowing waters. Taxa with high abundances (> 5 individuals per sample) were mainly limno- to rheophil, rheo- to limnophil, or rheophil. In the latter two classes the percentages of taxa with high abundances were higher than the percentages of taxa with low abundances. This was also the case for rheobiont and indifferent taxa (Fig. 1).

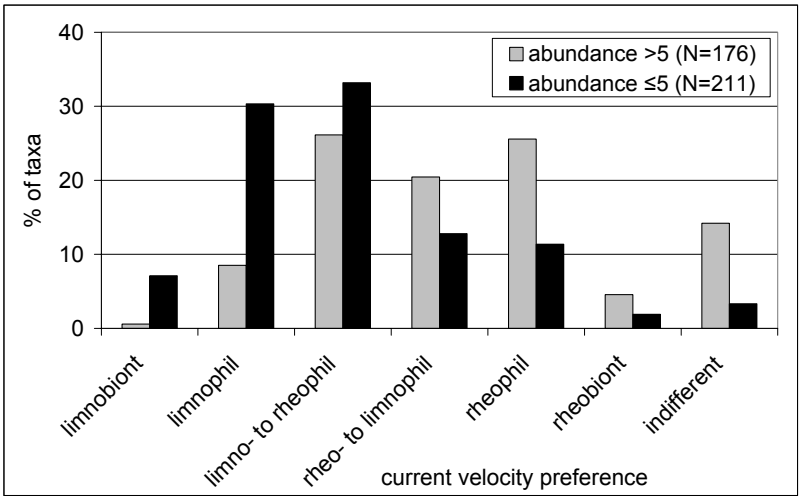

Fig. 1. Percentage of taxa with an average abundance ≤ 5 individuals per sample (N=339) and taxa with an average abundance > 5 individuals per sample (N=526) for all current preference types.

The mean scores for the saprobic valences were quite similar for both taxa with low abundances and taxa with high abundances (Fig. 2).

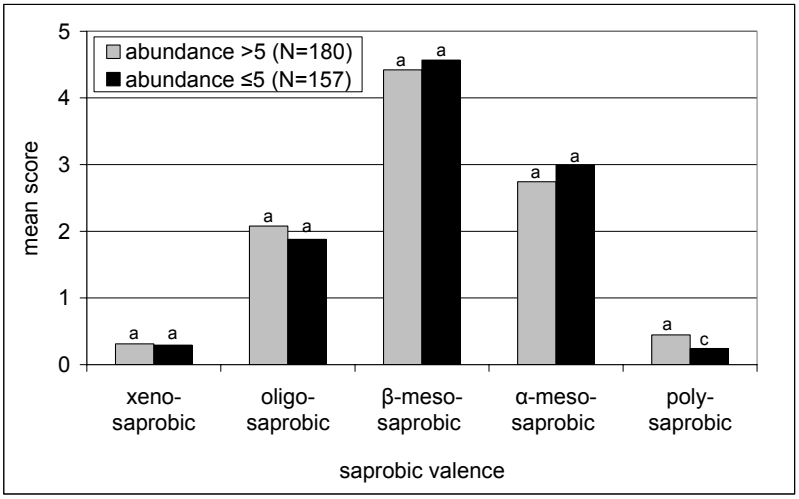

Fig. 2. Mean score for taxa with an average abundance ≤ 5 individuals per sample and taxa with an average abundance > 5 individuals per sample for all saprobic valences. Significance of differences between taxa with a low and taxa with a high abundance was tested with Students T-Test: a-a: no significant difference, a-b: significant difference (p=0.01), a-c: significant difference (p=0.05).

Differences between taxa with 5 or less individuals and more than 5 individuals per sample were small concerning microhabitat preferences (Fig. 3). The microhabitat lithal displayed the greatest difference between both abundance groups (1.0 for taxa with low abundances versus 1.7 for taxa with high abundances). Other typical stream habitats, such as psammal (sand) and akal (gravel) also showed higher mean scores for taxa with high abundances. Regarding the microhabitats pelal (mud), POM, phytal, and argyllal (silt), there were no significant differences between both groups of taxa.

Taxa with low abundances mainly belonged to the Coleoptera, Diptera, Trichoptera, and Heteroptera, The largest percentage of taxa with a high abundance belonged to the Diptera, followed by Trichoptera and Oligochaeta, respectively (Fig. 4).

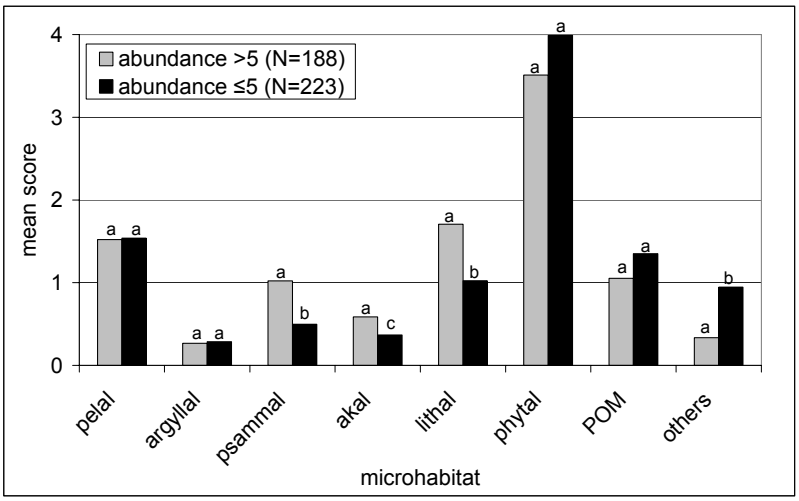

Fig. 3. Mean score for taxa with an average abundance ≤ 5 individuals per sample and taxa with an average abundance > 5 individuals per sample for all microhabitat preference types. Significance of differences between taxa with a low and taxa with a high abundance was tested with Students T-Test: a-a: no significant difference, a-b: significant difference (p=0.01), a-c: significant difference (p=0.05).

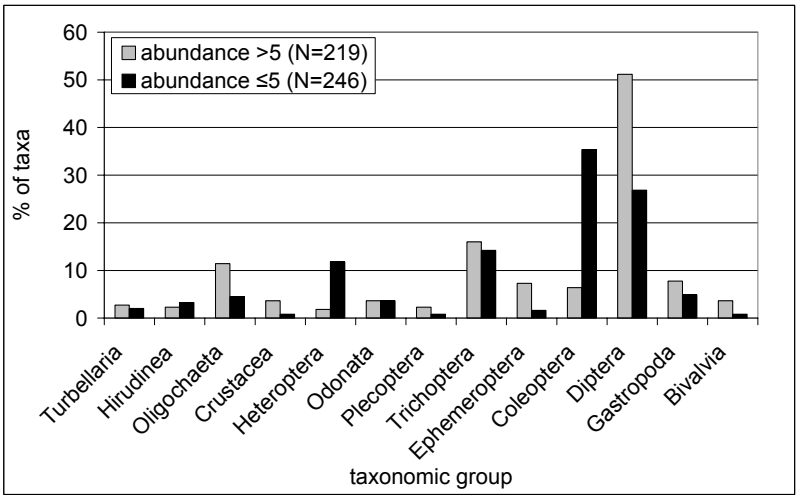

Fig. 4. Percentage of taxa with an average abundance ≤ 5 individuals per sample and number of taxa with an average abundance > 5 individuals per sample for all taxonomic groups.

Taxa with small distribution ranges

More than 40% of the taxa with a small distribution range within the 142 investigated samples were rheophilous (Fig. 5). Only a small percentage was rheobiont, occurring only in fast flowing parts of streams. Taxa with a large distribution range were

indifferent to current velocity or preferred standing or slowly flowing waters compared to taxa with a small distribution range.

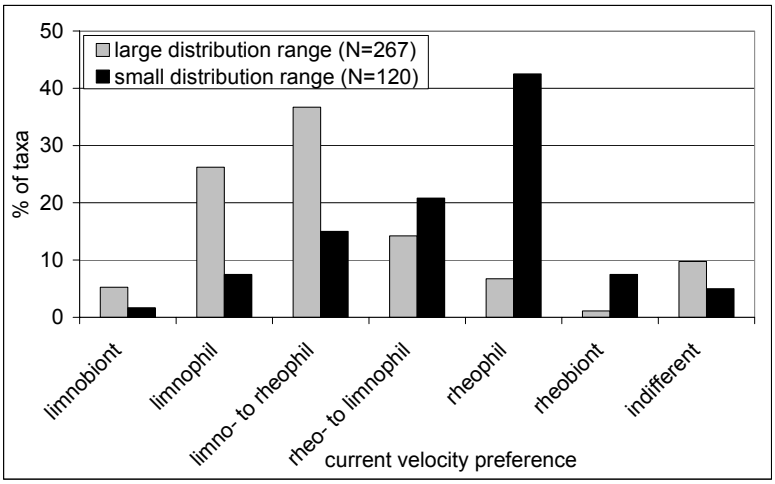

Fig. 5. Percentage of taxa with a small (very rare, rare, and uncommon) and large (common, very common, and abundant) distribution range for all current preference types.

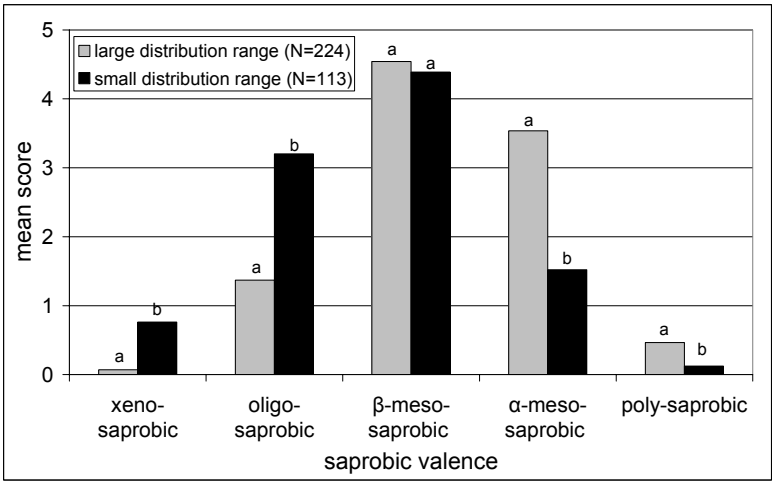

Fig. 6. Mean score for taxa with a small (very rare, rare, and uncommon) and large (common, very common, and abundant) distribution range for all saprobic valences. Significance of differences between taxa with a small and taxa with a large distribution range was tested with Students T-Test: a-a: no significant difference, a-b: significant difference (p=0.01), a-c: significant difference (p=0.05).

Taxa with small distribution ranges indicated more often xeno-saprobic and oligosaprobic stream conditions compared to taxa with large distribution ranges (Fig. 6). Taxa with large distribution ranges had higher preference scores for α-meso-saprobic and poly-saprobic conditions compared to taxa with small distribution ranges. In β-meso-saprobic streams, the mean scores for both groups of taxa were similar.

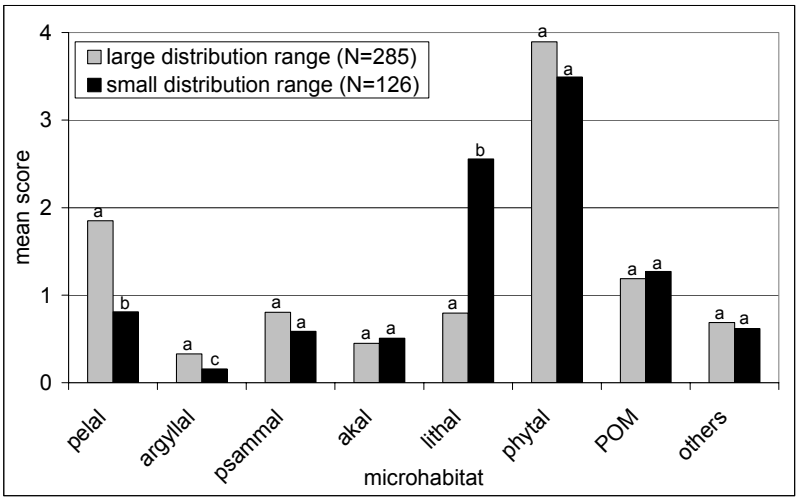

Fig. 7. Mean score for taxa with a small (very rare, rare, and uncommon) and large (common, very common, and abundant) distribution range for all microhabitat preference types. Significance of differences between taxa with a small and taxa with a large distribution range was tested with Students T-Test: a-a: no significant difference, a-b: significant difference (p=0.01), a-c: significant difference (p=0.05).

Taxa in both distribution range categories showed the highest preference for the microhabitat phytal (Fig. 7). All other microhabitats had relatively low scores. Two remarkable differences between taxa with small and large distribution ranges occurred: (1) taxa with small distribution ranges showed a much higher preference for lithal compared to taxa with large distribution ranges and (2) taxa with large distribution ranges displayed a higher mean score for pelal compared to taxa with small distribution ranges.

For both taxa groups, the highest percentages of taxa belonged to the Diptera (Fig. 8). Percentages of taxa with large distribution ranges were also high for Coleoptera (24%) and Trichoptera (10%). For taxa with small distribution ranges this was the other way around: Trichoptera had the second highest percentage (22%) followed by the percentage Coleoptera (13%). Other groups that showed a higher percentage for taxa with large distribution ranges were Heteroptera and Gastropoda.

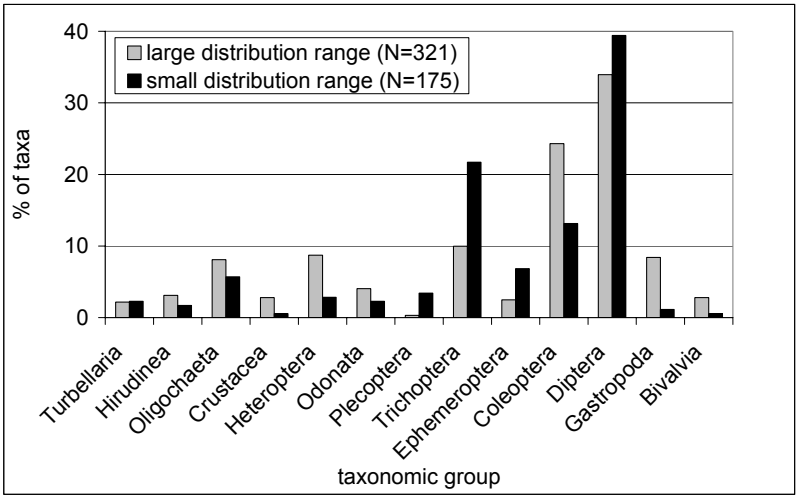

Fig. 8. Number of taxa with a small (very rare, rare, and uncommon) and large (common, very common, and abundant) distribution range for all taxonomic groups.

Rare taxa in relation to ecological quality classes

Taxa with low abundances

The distribution of taxa in the different abundance classes was similar for all ecological quality classes (Fig. 9). Only the number of taxa with high abundances was slightly higher in samples with higher ecological quality.

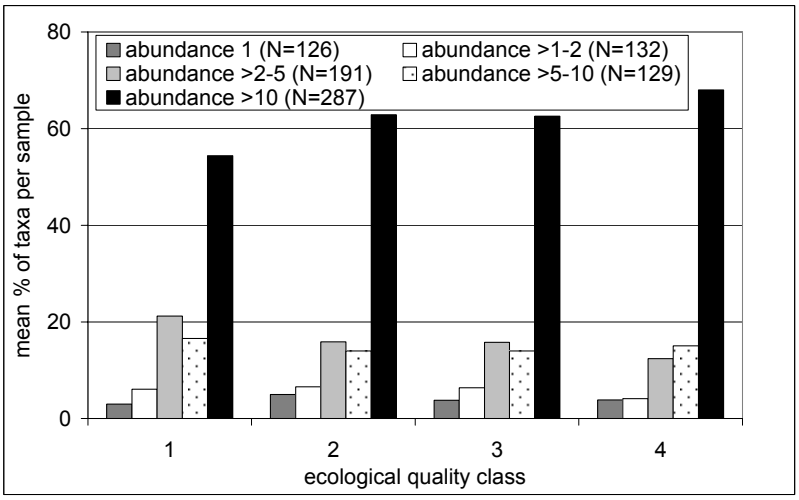

Fig. 9. Mean percentage of taxa within different abundance classes for the samples for each ecological quality class.

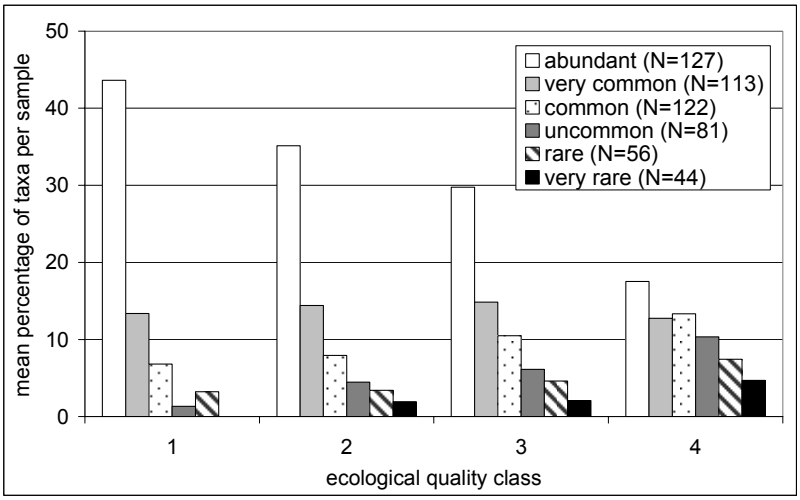

Fig. 10. Mean percentage of taxa per sample within different distribution classes for each ecological quality class.

Taxa with small distribution ranges

For taxa with small and large distribution ranges the results were different. The number of taxa with a large distribution range (abundant taxa) decreased if the ecological quality class became higher (Fig. 10). The number of very common taxa was similar for all ecological quality classes. For the other four distribution classes (very rare, rare, and uncommon common taxa) the number of taxa increased with higher ecological quality class. Table 3 also shows that the number of taxa with small distribution ranges (classes very rare, rare, and uncommon) was higher in samples that represented a higher ecological quality. Class 1 had only one such a taxon per sample, while the samples that belonged to ecological quality class 4 had almost 9 taxa that had a small distribution range on average per sample. The number of taxa with a small distribution range (very rare, rare, and uncommon) significantly differed between all ecological quality classes ($p<0.05$) except for class 2 and class 3 ($p=0.056$).

Table 3. Average number of taxa with a small distribution range (classes very rare, rare and uncommon) in the samples of each ecological quality class (calculated using all taxa).

ecological quality class	no. of samples	average no. of taxa	average no. of very rare, rare, and uncommon taxa
class 1	10	68	1.0
class 2	48	52	2.3
class 3	45	52	3.8
class 4	39	49	8.7

Excluding rare taxa from metric calculations

Data composition after excluding taxa with low abundances

In the total data set the mean number of taxa per sample was 53 and the mean number of individuals per sample was 3306 (Table 4). Excluding taxa with low abundances in particular reduced the number of taxa and to a lesser extent the number of individuals. Excluding all taxa from the samples which had an abundance of 10 or lower resulted in a reduction of about half of the taxa in the total data set and in a reduction of two-third of the number of taxa in the samples. However, the total number of individuals was only reduced by 3.5%.

Table 4. Characteristics for different selections of taxa data.

excluded taxa	total no. taxa left	mean no. taxa per sample	mean total abundance per sample
none	865	53	3306
abundance classes excluded			
abundance ≤ 1 individual per sample	739	41	3294
abundance ≤ 2 individuals per sample	647	33	3279
abundance ≤ 5 individuals per sample	526	24	3243
abundance ≤ 10 individuals per sample	424	17	3190
distribution classes excluded			
very rare	821	52	3285
very rare and rare	765	51	3208
very rare, rare, and uncommon	684	48	2988
very rare, rare, uncommon, and common	561	42	2671

Data composition after excluding taxa with small distribution ranges

Excluding taxa with small distribution ranges resulted in less extreme reductions of the total number of taxa and the mean number of taxa per sample (Table 4). Excluding very rare taxa led to a reduction of 44 taxa in the total data set but of only 1 taxon for the average number of taxa per sample. In this case, the number of individuals in the data set was reduced by only 21 on average per sample. If very rare, rare, uncommon, and common taxa were excluded from the data set, the total number of taxa was reduced by 31%, but the mean number of taxa per sample by only 21%. This is because many of the excluded taxa with a small distribution range occurred only in a limited number of samples. The total number of individuals per sample was strongly reduced by excluding taxa with small distribution ranges, which means that these taxa had high numbers of individuals.

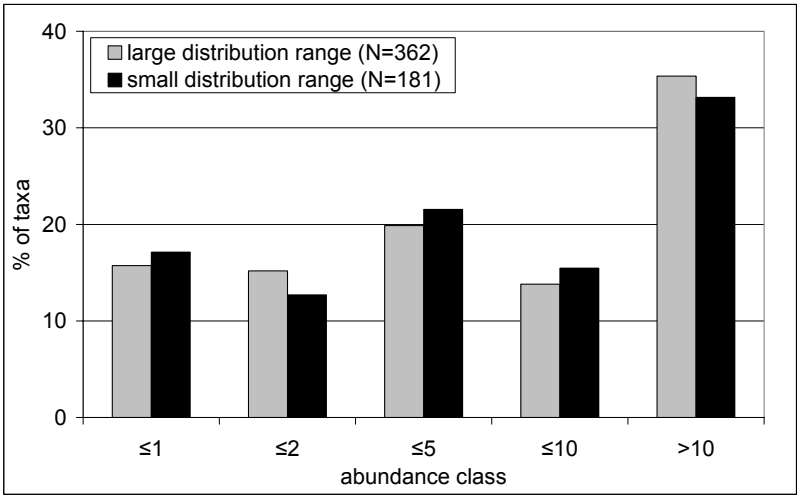

Fig. 11. Distribution of taxa with small and large distribution ranges over the abundance classes.

Fig. 11 illustrates that taxa with small distribution ranges may have high abundances at sites where they occur. Taxa with small distribution ranges and taxa with large distribution ranges were evenly distributed over all abundance classes. This means there is no relation between distribution range and abundance.

Multimetric results

Generally, a higher number of excluded taxa resulted in a higher number of samples that was classified differently compared to the original data set (Table 5). Excluding very rare, rare, uncommon, and common taxa resulted in 24 misclassifications. Excluding taxa with abundance ≤ 10 individuals per sample resulted in 27 different classifications. It is remarkable that using a taxa selection based on abundance classes led to higher final ecological quality classes, while using distribution classes for exclusion resulted in lower final ecological quality classes.

The metrics that resulted in a different ecological quality class if taxa were excluded from the data set were in most cases: the Saprobic Index, EPT-taxa / % Oligochaeta, % microhabitat pelal, and % current velocity preference rheophil. Also combined metrics, such as Gastropoda & EPT-taxa / Oligochaeta, resulted in different classifications if the results of the complete data set were compared to the results with the data set from which rare taxa were excluded.

Table 5. Classification of the samples using different exclusion data sets in comparison with the results using original data including all taxa.

	same class	no. of classes lower			no. of classes higher			total misclassified
		1	2	3	1	2	3	
abundance classes excluded								
≤ 1	128	0	0	0	8	1	0	9
≤ 2	118	2	0	0	13	0	0	15
≤ 5	101	3	0	0	17	0	0	20
≤ 10	77	5	0	0	22	0	0	27
distribution classes excluded								
very rare	142	0	0	0	0	0	0	0
rare and very rare	141	0	0	0	1	0	0	1
uncommon, rare and very rare	134	6	1	0	0	0	0	7
common, uncommon, rare, and very rare	116	16	2	0	6	0	0	24

Whether a metric result changed depended on the type of taxa that was excluded (Table 6). For example, the % Gastropoda only showed changes if many taxa with small distribution ranges were excluded. If taxa with low abundances were excluded nothing changed. It is obvious that excluding taxa with low abundances in a sample led to different results than excluding taxa with small distribution ranges. The largest differences were observed in metrics distinguishing ecological quality class 4. Excluding taxa with low abundances often resulted in the sample being assigned to class 4 instead of no class, which increased the final ecological quality class. Excluding taxa with a small distribution range often resulted in the sample not being assigned to a quality class instead of class 4, which resulted in a lower final ecological quality class.

Table 6. Changes in ecological quality classes for the metrics used in the multimetric index using the different taxa selections. The values in the table represent the number of sites that were misclassified (all sites were included). The change indicates if the respective metric scores for the class it separates from the others or not (no class). For further explanation of the metrics see Vlek *et al.* (2004).

Metric	change	abundance classes excluded				distribution classes excluded			
		≤1	≤2	≤5	≤10	very rare	rare and very rare	uncommon, rare, and very rare	common, uncommon, rare, and very rare
% grazers & scrapers / % gatherers-collectors & filterfeeders	no class → 1								
% grazers & scrapers / % gatherers-collectors & filterfeeders	1 → no class								
% river stretch hypopotamal	no class → 4				2				1
% river stretch hypopotamal	4 → no class				1			1	4
% microhabitat pelal	no class → 4	2	3	5	6				1
% microhabitat pelal	4 → no class							4	13
% current preference rheophil	no class → 4		1	2	2		1	1	2
% current preference rheophil	4 → no class						1	1	4
EPT-taxa / Oligochaeta	no class → 2	1	3	7	9			2	11
EPT-taxa / Oligochaeta	2 → no class	3	4	3	5				1
EPT-taxa / % Oligochaeta	no class → 2		2	3	4			2	6
EPT-taxa / % Oligochaeta	2 → no class								
% Gastropoda	no class → 1							1	4
% Gastropoda	1 → no class								
Gastropoda & EPT-taxa / Oligochaeta	no class → 3	1	1	4	5				3
Gastropoda & EPT-taxa / Oligochaeta	3 → no class	5	8	10	10				5
% hypopotamal EPT-taxa / Oligochaeta	no class → 3							1	1
% hypopotamal EPT-taxa / Oligochaeta	3 → no class			1	4		1	2	4
Saprobic index	no class → 4	1	1	3	6			1	1
Saprobic index	4 → no class							5	18

Discussion

The relation between abundance and distribution range

On the one hand rarity was defined using the abundance of taxa in the samples, on the other hand rarity was defined using distribution classes derived from the Dutch Macroinvertebrate Distribution Classification List. Apparently, there was no relationship between the abundance class and the distribution range of the taxa in Dutch lowland streams (Fig. 11). Taxa with a small distribution range had low or high numbers of individuals in the data set. This result differs from patterns that were described by a number of researchers (e.g., Hanski, 1997; Gaston, 1998). Generally, species in an assemblage that have higher numbers of individuals and/or higher local abundances are considered to have larger distribution ranges (Hanski & Gyllenberg, 1997; Gaston, 1998). This may be because generalist species, or species using ubiquitous resources are both locally common and widely distributed, whereas specialists (sensitive species) are constrained to a limited distribution range and tend to be locally uncommon (Brown, 1984; Hanski, 1991). Following Cao *et al.* (2001), a wide geographic distribution range means that a species has a broad tolerance to environmental conditions and probably high resistance to human disturbance in general. However, other data do not support this theory (Lawton, 1993). Gaston (1994) stated that species that are rare nowadays were the common ones formerly, when there was no disturbance by human activities. This fact could explain that some species with a small distribution range may still have high abundances in few suitable habitats that are still present.

The indicative value of rare taxa

Taxa with low abundances

Taxa with low abundances appeared not to be related to natural conditions in streams but more to habitats that are not specific for natural streams, such as pelal (mud) and phytal (vegetation). These microhabitats are more abundant in regulated and channelised streams. Taxa with low abundances indicated slowly flowing to standing water. Probably, they are either ubiquistic species, which can occur in a wide range of water types and habitats, or they are species that live under suboptimal circumstances and therefore have a limited number of individuals. Standing water taxa, for example, can live in streams that are channelised and/or regulated. In these streams, current velocity is reduced and often vegetation is present. Standing water taxa can also be typical inhabitants of lowland floodplains and side arms and migrate into the stream itself. On the other hand, taxa with high abundances had relatively high scores for high current velocity preference types and the microhabitat type lithal. Probably, these taxa inhabit their optimal habitat, which is a specific stream habitat.

Specific biological traits can also cause low abundances of taxa. Coleoptera, for example, include many species that never occur in large numbers, because many Coleoptera species are predators, which are often less abundant than prey species (Spencer, 2000). However, sampling methodology could also be the reason for low abundances in the data for groups such as Coleoptera and Heteroptera. Most of these taxa live on the water surface or between vegetation and can easily escape the sampling net because they are fast swimmers.

In our study, the percentage of the taxa with low abundances did not indicate the ecological quality of a site. Probably, at sites within each ecological quality class there are taxa that occur only in low numbers. Part of these taxa overlap between ecological quality classes and have low abundances because of their species traits (these taxa are not influenced by human disturbance), others however have low numbers because they do live under suboptimal conditions (these are different taxa in the different ecological quality classes).

Taxa with small distribution ranges

Generally, throughout this study, taxa with small distribution ranges appeared to be more indicative for unimpacted streams than taxa with low numbers of individuals. This was shown by a strong relation between the number of taxa with small distribution ranges and high ecological quality classes and by their indication of (fast) flowing water and typical natural stream habitats such as lithal, akal, and psammal.

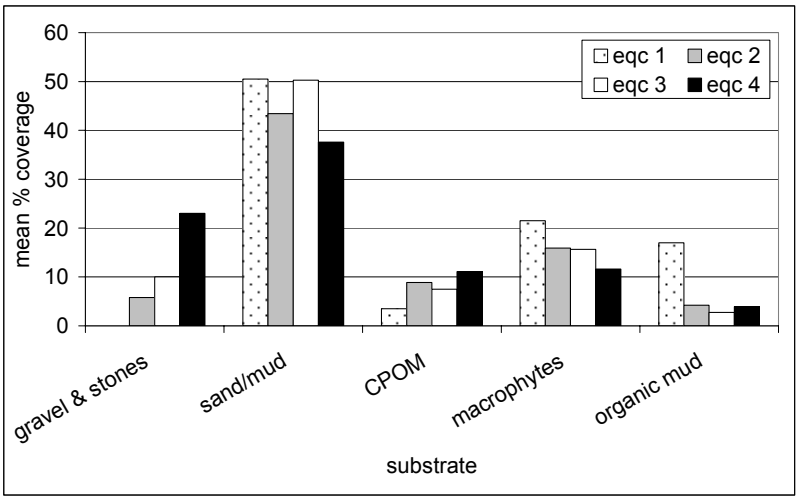

Fig. 12. Mean percentage of coverage of the main substrate classes for the streams within the four ecological quality classes (eqc).

This pattern is confirmed by Fig. 12, which shows that the sites with high ecological quality (class 4) have a high mean coverage of gravel and stones (lithal and akal) and that the sites in ecological quality class 1 have a relatively high percentage of organic mud.

The indication of xeno- to β-mesosaprobic streams by the taxa with small distribution ranges was remarkable as was also the indication of α-mesosaproby and poly-saproby by taxa with large distribution ranges. Taxa with small distribution ranges seem to be limited to unimpacted, clean waters. Probably, they are susceptible to organic pollution besides their vulnerability for disturbance of typical stream habitats. Several researchers have shown that rare species are one of the most reliable indicators of degradation and that they are therefore very important in biological assessment and conservation of biodiversity (Lyons *et al.*, 1995; Cao *et al.*, 1998, Lenat & Resh, 2001). This was confirmed by our study. Generally, species that are widely distributed are associated with unstable and disturbed habitats more frequently than species with small distribution ranges (e.g., Thomas, 1991; Gaston, 1994; Rakocinski *et al.*, 1997).

Conservation and restoration of habitat diversity and avoiding organic pollution are major criteria for biodiversity conservation in streams. Including species with small distribution ranges in assessment and monitoring enables water and nature managers to detect any disturbances faster and to indicate the type of disturbance (Cao *et al.*, 2001). Species with small distribution ranges often disappear first after disturbance or pollution and reappear slowly. Hence, the disappearance of these species may indicate subtle changes in the environment. They are therefore important in detecting changes from good towards moderate ecological status in an early stage. Species with small distribution ranges can also be used as indicators that point out the effect of restoration measures and whether the change really resulted in an ecological improvement. Only if these species of the community have also returned or increased in density the whole ecological community is restored.

This study clearly showed that the number of species with small distribution ranges is related to high ecological quality. Therefore, the number of species with small distribution ranges is a useful metric in monitoring restoration projects and assessment of habitat diversity and/or water quality.

The effect of excluding rare taxa on ecological assessment using the AQEM method

Excluding taxa with low abundances

Excluding taxa with low abundances resulted in a strong reduction of the number of taxa per sample but in a small reduction of the number of individuals per sample. For water managers, excluding taxa with low abundances is a cost-effective and thus an interesting method when assessing stream quality, e.g., using the AQEM method.

First, the number of taxa, which need to be identified, is reduced; second, it is less time consuming to identify many individuals of one taxon, than few individuals of many taxa. The easiest way to exclude taxa with low abundances is to subsample (Barbour & Gerritsen, 1996). Another way is to exclude taxa of which it is possible to see by eye that there are only a few individuals of these taxa.

However, the ecological quality class was overestimated if taxa with low abundances were excluded from the data. This is problematic, especially, if streams with a moderate ecological status are assessed as having a good ecological status. In these cases there is ecological damage and measures would be necessary to reach good ecological status. However, the degradation is not recognised and no measures will be taken. In these cases the requirements of the Water Framework Directive clearly cannot be met. Therefore, it can be misleading to exclude taxa with low abundances from the AQEM method.

There are two explanations for the occurrence of misclassifications. First, part of the taxa with low abundances showed preferences for pelal and slowly flowing to standing water. This indicates that the streams in which they occur are channelised and regulated. If these taxa are excluded a higher ecological quality class is the result. However, only the metric '% microhabitat pelal' resulted in some misclassifications. This metric scored quality class 4 instead of no class, which resulted in an overestimation of the ecological quality class.

Second, and even more important, excluding taxa with low abundances did substantially shorten the list of taxa to be used in the assessment system. Cao *et al.* (1998) concluded that this could lead to an underestimation of the difference between undisturbed and impacted sites. Removal of taxa with low abundances reduced the number of taxa and therefore, the expected range of taxa richness is smaller (Cao *et al.*, 2001). In our study, the metrics that are based on taxon numbers, such as EPT-taxa / Oligochaeta, showed a relatively large number of misclassifications. Therefore, to avoid a high number of misclassifications if taxa with low abundances are excluded, metrics should be chosen that focus on the numbers of individuals instead of the number of taxa. For example, EPT-taxa / % Oligochaeta (percentage of individuals) could be used instead of EPT-taxa / Oligochaeta (numbers of taxa). There were fewer miscalculations using the first, rather than the latter metric.

In conclusion, using the AQEM method, excluding taxa with low abundances resulted in overestimation of the ecological quality class. However, it is the question what the result would be if the AQEM system was developed without the low abundant species. Probably, there would have been less misclassifications if taxa with low abundances were excluded from assessment. However, taxa with low abundances appeared to be indicative for hydro morphological degradation and therefore the AQEM system would have been less compatible if these taxa were excluded from the development of the system.

Excluding taxa with small distribution ranges

Excluding taxa with small distribution ranges resulted in a minor reduction of taxa per sample but in a large reduction of the number of individuals per sample. Therefore, for water managers, excluding taxa with small distribution ranges is less effective to decrease the costs than excluding taxa with low abundances. To skip taxa with small distribution ranges from sorting and identification processes is to mark the taxa in identification keys. In many cases identification can be simplified if rare species are excluded.

The ecological quality was underestimated in most cases when taxa with small distribution ranges were excluded from the data. For water management this would lead to higher costs, because measures have to be taken to improve the quality of a stream, while in fact the quality is already good. The effect of excluding taxa with small distribution ranges was largest for the Saprobic Index. The Saprobic Index resulted in lower ecological quality classes when species with small distribution ranges were excluded. The high number of species with small distribution ranges indicating oligosaprobic conditions can explain this. Saprobic indices have proven to be good measures for organic pollution (Zelinka & Marvan, 1961, 1966; Sládeček, 1973) and they often show clear differences between natural and degraded streams. Apparently, species that have low distribution ranges are limited to oligosaprobic streams, which have become rare in the Netherlands. Gaston (1994) already stated that many species that were more common in times when human disturbances did not yet occur became rare because of impact of human activities. Other metrics that resulted in a high number of misclassifications were % microhabitat pelal and EPT-taxa / Oligochaeta. Taxa with small distribution ranges indicated other habitats, such as lithal and phytal. Excluding these, results in a relatively large number of taxa with a preference for the microhabitat pelal. The metric EPT-taxa / Oligochaeta was probably influenced by excluding oligochaete taxa with small distribution ranges but high abundances.

The effect of excluding taxa with small distribution ranges would probably have been larger if reference sites (completely undisturbed sites) were included in the data. Apparently, taxa with small distribution ranges indicated undisturbed conditions. Metrics that separated ecological quality class 4 from the others, were most influenced by excluding the taxa with small distribution ranges. Nijboer *et al.* (2004) showed that including reference sites (ecological quality class 5) from other countries in the Dutch data set resulted in higher values for most of the metrics. Also, more rare species were included in the reference sites.

Because taxa with small distribution ranges are related to undisturbed conditions it would have been no option to develop the AQEM system without these taxa. Including rare taxa results in longer gradients in the data and therefore a more clear distinction between ecological quality classes is possible.

In the AQEM method habitats that cover less than 5% of the surface area were not included in the samples. This means that taxa that are rare because they occupy a rare habitat were not included. However, this has no effect on the assessment results as long as these specific habitats are not related to undisturbed conditions.

Conclusion

From our study we can conclude, that excluding taxa with low abundances or small distribution ranges should be avoided for ecological assessment purposes. Excluding taxa with low abundances strongly reduced the number of taxa in the samples. Ecological assessment of the samples without taxa with low abundances resulted in assigning a higher ecological quality class, because some taxa with low abundances indicated morphological degradation and the number of taxa in the samples was strongly reduced.

Excluding taxa with small distribution ranges especially reduced the number of individuals in the samples. The samples were then classified into a lower ecological quality class, because many of the taxa with small distribution ranges indicated special habitat conditions or unimpacted sites. Taxa with small distribution ranges are very indicative for high ecological quality and it is therefore recommended to add a metric using the number of taxa with small distribution ranges in the assessment system.

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5 Ecological assessment of aquatic ecosystems: Taxa richness is not the item, rare taxa are

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Abstract

Assessment of the ecological quality of fresh waters using macroinvertebrate communities relies on the variables chosen. The number of rare taxa and taxa richness were evaluated as potential variables for ecological assessment of streams and channels. Distribution classes were applied to a data set with macro-invertebrate samples of streams and a data set with samples of small channels. Taxa richness, the number and fraction of rare taxa (occurring in less than 1.5% of the water bodies all over the Netherlands) were counted per sample. The average number of rare taxa per sample appeared to be low, particularly in the channels. Subsequently, the relationship between taxa richness, the number of rare taxa, and the fraction of rare taxa and environmental variables were studied. The number of rare taxa and the fraction of rare taxa were both positively related to environmental variables indicating naturalness and negatively related to variables indicating human impact. This relationship was not found for taxa richness, which was more related to habitat variables. To exclude the influence of habitat variables taxa richness and the number of rare taxa of sites with similar habitat variables but different ecological quality (4 classes: bad, poor, moderate, and good ecological quality) were compared. The number of rare taxa increased from bad to good ecological quality. Taxa richness showed the highest value at sites of poor and moderate ecological quality for streams and channels, respectively. In conclusion, rare taxa can be a useful parameter in ecological assessment of freshwaters, because their presence indicates natural circumstances. Standard macroinvertebrate sampling as it is carried out in Europe is sufficient to collect enough rare taxa to be able to determine ecological quality. Taxa richness should be used carefully in ecological assessment, because it indicates typological differences rather than ecological quality, and the change of taxa richness due to human impact strongly depends on the type of stressor. Therefore calibration of the response of taxa richness to stressors should be carried out for each water type separately before using it as a community variable in ecological assessment.

Keywords: macroinvertebrate, rare taxa, taxa richness, assessment, ecological quality, freshwater, distribution

Introduction

Biodiversity of macroinvertebrates is an important issue in the conservation of aquatic ecosystems. Two features of biodiversity, taxa richness and the number of rare taxa can be useful in ecological assessment. However, it is still unclear whether these variables are only related to human impact or that habitat variables also play a role.

A large number of biological indicator metrics and assessment systems have been developed for the evaluation of the ecological quality of fresh waters (reviews: Metcalfe, 1989; Verdonschot, 2000). Assessment methods consider either the complete macroinvertebrate community, e.g., RIVPACS (Wright, 2000) and EKOO (Verdonschot and Nijboer, 2000) or include a combination of community aspects, such as indicator taxa (Kolkwitz and Marsson, 1908, 1909; Sládeček, 1973), taxa richness, or number of Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxa (Lenat, 1988), or a combination of metrics (Karr *et al.*, 1986; EPA, 1988). In many of the assessment systems taxa richness is included as one of the metrics. Particularly since biodiversity has become a major issue, taxa richness appeared to be a practical indicator of biodiversity. Many studies have shown that taxa richness is a good metric for environmental assessment studies and that human impact is indicated by reduced taxa richness (Rapport, 1991; Resh, 1994; Sandin and Johnson, 2000; Resh *et al.*, 2000).

However, other authors have suggested that high taxa richness is not always related to unimpacted sites (reference conditions). Rivers with high taxa richness are not necessarily important for conservation and rivers with low taxa richness can be interesting for conservation purposes (Boon, 2000). Relatively natural rivers in some locations e.g., with harsh environments (Wright *et al.*, 1998) can be taxa-poor. Thus, before taxa richness is used as a metric in the assessment of ecological quality, the relation between taxa richness and environmental conditions should always be studied for the water type (habitat) in question.

Another community variable that may be useful in ecological assessment is the presence of rare taxa. However, rare taxa are only sometimes used as indicators of good ecological status, for example in the scoring system SERCON, which has been developed for the evaluation of river conservation, in which rarity is one of the attributes (Boon *et al.*, 1997). In population and community ecology rare taxa are regarded as those taxa that have a low abundance and/or a small distribution range (Gaston, 1994). Nijboer and Verdonschot (2004) used the frequency of occurrence of taxa as a criterion for the classification of distribution ranges for Dutch macroinvertebrates. They defined six classes (very rare 0-0.15%, rare >0.15-0.5%, uncommon >0.5-1.5%, common >1.5-4%, very common >4-12%, and abundant >12% of the sites) and showed that 62% of the Dutch macroinvertebrate taxa were classified as uncommon, rare, or very rare, occurring at less than 1.5% of the sites. Nijboer and Schmidt-Kloiber (2004) studied both distribution range and abundance as criteria for rarity and these authors showed that taxa with small distribution ranges

indicated a high ecological quality class rather than taxa with low abundances. Cao *et al.* (2001) concluded that including rare taxa in assessment and monitoring enables water and environmental managers to detect disturbance faster and to indicate the type of disturbance.

On the other hand, Nijboer and Schmidt-Kloiber (2004) observed that habitat variables may influence the occurrence and number of rare taxa at a site. Rare taxa (with small distribution ranges) are often related to specific habitats because (1) their habitat represents extreme circumstances which are rare, and to which only few taxa have become adapted (in less extreme habitats these taxa are outcompeted) and/or (2) their habitat has become rare by human impact. In both cases, rare taxa can be used to indicate special habitats worth conserving. Gaston (1994) suggested that small distribution areas of taxa that are considered to be rare nowadays are in great part a result of human activities. In the case that the habitat of a taxon has become rare because of human impact, the presence of the rare taxon is a good indicator of ecological quality (Lyons *et al.*, 1995; Nijboer and Schmidt-Kloiber, 2004). Common taxa, on the other hand, are often widely distributed (Gaston, 1994) and associated with unstable and disturbed habitats more frequently than rare taxa (e.g., Thomas, 1991; Rakocinski *et al.*, 1997). To better use of the number of rare taxa at a site it is necessary to know to which environmental variables the number of rare taxa is related and whether it indicates high ecological quality rather than extreme conditions.

In conclusion, both taxa richness and the number of rare taxa may be good indicators for ecological quality. However, both seem to be related to habitat variables as well. This can be a problem if assessment of ecological quality must be comparable between countries or water types as is the case in the European Water Framework Directive (European Commission, 2000). In this study three research questions were addressed using national and regional databases including different types of flowing and still waters:

1. Is there a difference between flowing and still waters concerning taxa richness and the number of rare taxa at a site?
2. Are taxa richness and the number of rare taxa at a site related to habitat variables or variables related to human impact?
3. Are taxa richness and the number of rare taxa at a site related to the ecological quality if sites of the same water type are compared?

Methods

Data collection

Stream and channel data were collected from water district managers all over the Netherlands. Macroinvertebrates were sampled by the water district managers using a standard macroinvertebrate sampling protocol (Verdonschot, 1990). In summary, a sample of 1.5 m^2 was taken with a pond dipnet. The sample was collected from the habitats present in the stretch of the stream or the channel. The stream data set contained 2242 macroinvertebrate samples. Environmental variables were recorded for 563 of these samples, also following a standard procedure (Verdonschot, 1990). Most streams were slow flowing sandy lowland streams. The channel data set contained 6127 macroinvertebrate samples. The channels included in the data set were less than 15 m wide. These channels are often used for drainage of agricultural area. The aquatic ecosystem in these channels is comparable with other standing waters, such as ponds or oxbow lakes. Environmental variables were measured in 410 channels. In both data sets animals were identified to species level where possible. Identifications were carried out by the water district managers.

Nijboer and Verdonschot (2004) assigned distribution classes based on the number of occurrences in the Netherlands to 1544 macroinvertebrate taxa to identify their rarity. They defined six distribution classes based on the percentage of sites all over the Netherlands (in a database of samples of 7608 sites) in which a taxon occurred (very rare 0-0.15%, rare >0.15-0.5%, uncommon >0.5-1.5%, common >1.5-4%, very common >4-12%, and abundant >12% of the sites). In this classification abundance measures were not used as a criterion. The list of 1544 taxa and their distribution classes was used in our study to assign the distribution classes to all taxa in the stream and channel data. In total, the data set with streams contained 943 taxa with a distribution class; the database with channels contained 839 taxa with a distribution class. More than 90% of the taxa in both data sets were species. Other taxa were mainly species groups or genera.

Environmental variables

A total of 54 environmental variables were available for the stream data, and 37 for the channel data. Clustering (using a non-hierarchical clustering program called FLEXCLUS (Van Tongeren, 1986) and ordination (Canonical Correspondence Analyses with forward selection using the program CANOCO (Ter Braak and Šmilauer, 2002) were carried out to select the variables that showed the highest correlation with the variation in the macroinvertebrate data. This resulted in a selection of 19 environmental variables for the stream data and 20 for the channel data (Verdonschot and Nijboer, 2004; Nijboer *et al.*, 2003, respectively). The selected variables, which were different for streams and channels, are listed in Table 1.

Table 1. Description of environmental variables selected for streams and channels.

variable	unit	description for	
		streams	channels
width	m	water surface at sampling site	water surface at sampling site
depth	m	at deepest part of the transversal profile	in middle of the channel
in natural area	0/1	if situated in nature reserve score 1	if situated in nature reserve score 1
natural profile	0/1	if natural transversal profile score 1	not included
meandering	0/1	if meandering length profile score 1	not included
presence of dams	0/1	if dams are present within 500 m score 1	not included
permanency/ inter-mittency	0/1	if permanent score 1	if intermittent score 1
current velocity	m/s	at sampling site	not included
shade	%	% coverage	not included
silt	%	% coverage	not included
sand	%	% coverage	if main soil type score 1
pH	-	measured once	annual mean
conductivity	µS/cm	measured once	annual mean
chloride	$mg.l^{-1}$	annual mean	annual mean
ammonium	$mgN.l^{-1}$	annual 90 percentile	annual mean
nitrate	$mgN.l^{-1}$	annual 10 percentile	annual mean
Kjeldahl nitrogen	$mgN.l^{-1}$	annual 90 percentile	not included
total nitrogen	$mgN.l^{-1}$	not included	annual mean
total phosphate	$mgP.l^{-1}$	annual 90 percentile	annual mean
oxygen content	$mg.l^{-1}$	annual 10 percentile	not included
floating vegetation	%	not included	% coverage in 100 m reach
filamentous algae	%	not included	% coverage in 100 m reach
emergent vegetation	%	not included	% coverage in 100 m reach
submerged vegetation	%	not included	% coverage in 100 m reach
groundwater seepage	0/1	not included	groundwater seepage present score 1
water inlet	0/1	not included	if water is let in, score 1
soil type	0/1	not included	peat, clay, or sand, dominant type scores 1

To make distributions of environmental variables more symmetric, all percentages were transformed to the empirical logit scale. Other continuous variables, except for pH and the 10^{th} percentile of the oxygen content, were log transformed adding a small constant if required. For the stream data a proportion of 19% of the values for the selected environmental variables were missing, while the channel data only missed 4% of the values. The imputation model of Schafer (1997) was used to

once-only impute the missing values. The imputation model was carefully selected using statistical testing with the MIX software (Schafer 1997). An important variable in the imputation model is a grouping of the samples based on the macro-invertebrate data. This reflects the fact that relations between environmental variables were different for the different groups. The imputation model was used to replace missing values in a sample by their conditional mean given the observed values in the samples of the macroinvertebrate sample group.

Taxa richness and number of rare taxa in flowing and still waters

Taxa richness and the number of taxa in each distribution class were counted for the complete stream and channel data sets as well as for each sample. The number of rare taxa was counted adding all taxa with distribution classes uncommon, rare, and very rare, because these classes showed the largest differences in numbers related to ecological quality for a small stream data set (Nijboer and Schmidt-Kloiber, 2004).

Taxa richness and number of rare taxa in relation to environmental variables

To answer the question whether taxa richness and the number of rare taxa at a site are related to environmental variables indicating human impact rather than habitat variables the whole stream and channel data sets were analyzed separately. There was no further selection of types within both data sets. Thus, sites of different types (habitats) and sites of different ecological quality were included.

For each stream and channel site taxa richness and the number of rare taxa were used. Also the fraction of rare taxa (number of very rare, rare, and uncommon taxa divided by the total number of taxa) was determined for each sample. The relations between the number of rare taxa, the fraction of rare taxa and the total number of taxa and the observed environmental variables were studied.

The relations between the total number of taxa, the number of rare taxa and environmental variables were investigated using Poisson regression with overdispersion (McCullagh and Nelder, 1989). The analyses for the fraction of rare taxa in relation to the environmental variables were carried out using logistic regression, also with overdispersion (McCullagh and Nelder, 1989). In all regressions the samples were weighted according to the number of non-missing environmental variables in the respective sample. This compensates for the imputation of missing data so that samples with many missing variables are downweighted. A screening analysis of environmental variables, similar to the approach adopted by Brown (1976), was performed. In a screening analysis all environmental variables are subjected to two statistical tests: a marginal and a conditional test. In the marginal test the relation between the response and a variable on its own is tested for statistical significance. In the conditional test the relation between the response and a variable is tested after

correction for the effects of all other variables. In practice the conditional test is performed by removing a variable from the model with all variables. Marginal and conditional significance can be very different due to collinearity between variables.

Finally, for each response the best model was selected. This was done by fitting all possible models, and to select the most concise model with smallest mean deviance and with p-values less than 0.01 for all included variables. The fitting of all possible models was done iteratively with groups of maximally 12 variables. Interactions between variables or quadratic effects were not considered in this analysis.

Taxa richness and number of rare taxa in relation to ecological quality

To relate taxa richness and the number of rare taxa at a site to ecological quality the influence of habitat variables was excluded by selecting sites from both the stream and channel data sets within one reference type. From the stream data set only sites from headwater streams which were smaller than 3 m and had a current velocity < 0.3 m/s were used. These class boundaries resulted from earlier clustering and ordination analyses (Verdonschot and Nijboer, 2004). Within this stream type environmental variables indicating human impact were the major explaining variables resulting from the ordination analyses.

From the channel data intermittent channels, brackish channels (chloride concentration > 600 mg/l), and channels wider than 8 m were excluded. The remaining small, permanent, fresh water channels only showed differences in ecological quality, expressed by environmental variables indicating human impact resulting as major variables from ordination analyses (Nijboer *et al.*, 2003).

Each site within the two data sets was classified using the ecological quality classes defined by the European Water Framework Directive (European Commission 2000): bad ecological quality (class 1), poor ecological quality (class 2), moderate ecological quality (class 3), and good ecological quality (class 4). There were no sites which could be classified as having a high ecological quality (class 5). To classify the sites, the AQEM assessment system was used conform Hering *et al.* (2004). This system calculates the ecological quality of a site using the complete taxa composition in a large number of metrics combined in a multimetric for each country and water type.

Finally, the number of taxa and the number of rare taxa (with distribution classes uncommon, rare, and very rare) were counted for each site and the means were calculated for each ecological quality class. The differences between ecological quality classes were tested using the Students t-test.

Results

Taxa richness and number of rare taxa in flowing and still waters

The total number of taxa in the stream data set was higher than in the channel data set (943 versus 839 taxa). The total numbers of taxa in stream and channel samples were similar (means of 45.6 and 44.9 taxa for channels and streams, respectively).

In the streams and channels sampled, the numbers of uncommon, rare and very rare taxa were about half (469 and 369 taxa for streams and channels, respectively) (Fig. 1) of the total number of uncommon, rare and very rare taxa that are recorded for the Netherlands (956 taxa) (Nijboer and Verdonschot, 2004). On the other hand, 93% of all common, very common, and abundant taxa of the Netherlands were collected.

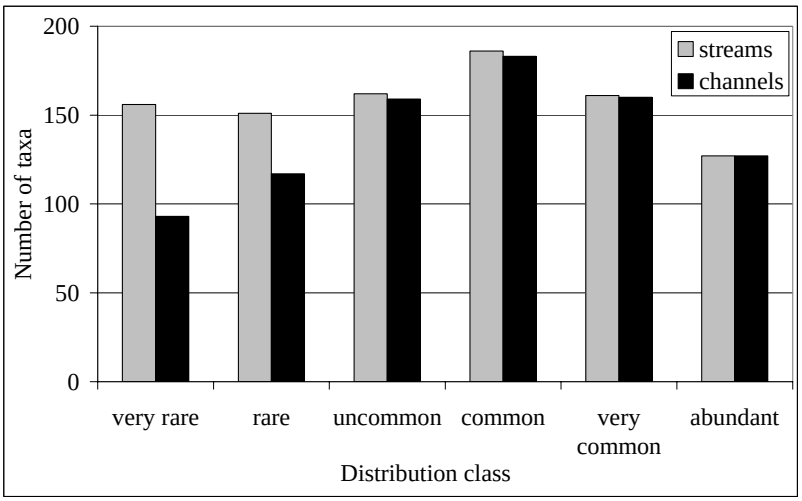

Fig. 1. Number of taxa observed in stream and channel data in each distribution class (very rare = 0-0.15%, rare >0.15-0.5%, uncommon >0.5-1.5%, common >1.5-4%, very common >4-12%, and abundant >12% of the sites in the data set of 7608 sites used by Nijboer & Verdonschot (2004)).

Although many rare taxa were present in the data sets, the numbers of rare taxa (including the distribution classes uncommon, rare, and very rare) per sample were low (Fig. 2). Most of the samples contained none or only one or two rare taxa. There were few samples that contained more rare taxa. Only 7.5% of the channels included more than 2 rare taxa. Only 15% of the streams contained more than 4 rare taxa. On average 2.2 rare taxa were found in a stream sample and 0.8 in a channel sample.

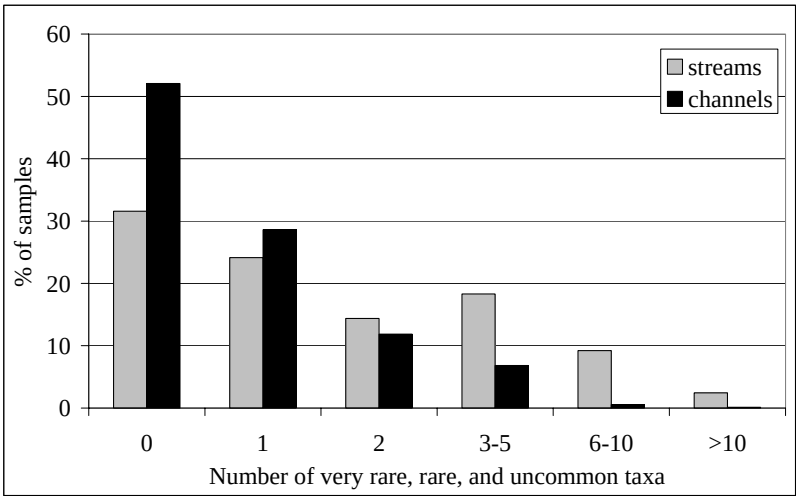

Fig. 2. Numbers of very rare, rare, and uncommon taxa in stream and channel samples.

All abundant taxa occurred in both streams and channels (Table 2). Very common, common, and uncommon taxa were mostly taxa that were observed in both water types. Rare and very rare taxa were more often related to either streams or channels.

Table 2. Number of species in each rarity class occurring in either streams or channels or in both streams and channels.

distribution class	number of taxa only in streams	number of taxa only in channels	number of taxa in both streams and channels
very rare	105	42	51
rare	73	39	78
uncommon	28	25	134
common	12	9	174
very common	6	5	155
abundant	0	0	127

Taxa richness and number of rare taxa in relation to environmental variables

The results of the marginal and conditional tests of the environmental variables indicate that in streams taxa richness was related to other variables than the number and fraction of rare taxa (Table 3). Variables that were positively related to taxa richness were negatively related to the number and fraction of rare taxa. Only for sand and pH this was not true, but these variables were not significant in the marginal tests in some cases. For taxa richness all marginal tests, except for total phosphate, were

significant (Table 3). However, due to collinearity of the environmental variables, only the conditional tests for silt, depth, width, pH, chloride and conductivity were significant. Variables that indicate unimpacted sites, such as natural area, natural profile, meandering were negatively correlated with taxa richness. Variables that indicate human impact, e.g., the presence of dams, chloride, ammonium and total phosphate levels were positively correlated with taxa richness. High taxa richness appeared to be correlated to the larger streams, which are more often regulated and canalized. Current speed is low in these streams and therefore negatively correlated to the total number of taxa. Model selection resulted in a model with the variables width, depth, pH, conductivity and total phosphate (Table 4).

Natural profile, meandering and natural area (Table 3) showed a positive relationship with the number of rare taxa. Variables indicating human impact, such as the presence of dams, a high chloride level, high total nitrogen (Kjeldahl) content and silt showed a negative correlation with the number of rare taxa. Only sand was not significant in the marginal test. In the conditional test, many variables were not significant anymore due to collinearity. Natural profile, pH, chloride, conductivity and nitrate were included in the selected model (Table 4). The results for the fraction of rare taxa (Table 3) were similar to those for the number of rare taxa. The best fitting model included the same variables as the model for the number of rare taxa, but it additionally included width and ammonium content (Table 4).

Also for channels, taxa richness was related to other variables than the number and fraction of rare taxa (Table 5) but the differences were smaller. Width, pH, conductivity, clay, intermittency, chloride, ammonium, nitrate, total nitrogen and total phosphate concentrations were negatively related to taxa richness as well as number and fraction of rare taxa. But in all cases, except for conductivity, the regression coefficients were stronger negative for the number and fraction of rare taxa, indicating that the negative influence of these environmental variables is stronger for the number and fraction or rare taxa than for taxa richness. The variables 'in nature reserve', groundwater seepage, peat, sand and submerged vegetation were positively related to taxa richness and the number and fraction of rare taxa. Also for these environmental variables, regression coefficients were higher for the number and fraction of rare taxa, indicating stronger relationships. Remarkable differences were a positive relation between the impact factor water inlet and taxa richness but a negative relation with the number and fraction of rare taxa. Also floating vegetation (often duckweed) had a positive relationship with taxa richness but a negative relation with the number and fraction of rare taxa. Intermittency had a negative relation with the total number of taxa but a positive relation with the number and fraction of rare taxa.

The results of the univariate regression analyses (Table 5) showed that for taxa richness almost all variables were significant if they were tested separately (p marginal <0.05). Only pH, emergent vegetation, sand, intermittency and groundwater seepage were not significant. For the conditional tests more variables were not significant,

because they were correlated with others, e.g., ammonium concentration. The selected model consisted of 7 variables (Table 6); depth, chloride, conductivity, pH, total phosphate, submerged vegetation and water inlet. In each of the models that were tried, the variables; chloride, conductivity, and pH were included (Table 6). The other variables varied in alternative models with comparable fit.

Table 3. Screening results of the environmental variables for taxa richness, number and fraction of rare taxa in streams, including the estimated regression coefficient of the marginal model (beta), the p-value of the marginal test (p-marg) and of the conditional test (p-cond).

	taxa richness			number of rare taxa			fraction of rare taxa		
variable	p-marg	p-cond	beta	p-marg	p-cond	beta	p-marg	p-cond	beta
depth	0.000	0.000	0.318	0.000	0.019	-0.230	0.000	0.727	-0.604
width	0.000	0.008	0.318	0.000	0.240	-0.294	0.000	0.000	-0.714
in natural area	0.000	0.274	-0.308	0.000	0.784	0.709	0.000	0.600	1.111
natural profile	0.000	0.863	-0.255	0.000	0.000	1.163	0.000	0.000	1.536
meandering	0.000	0.154	-0.223	0.000	0.086	1.103	0.000	0.280	1.435
permanency	0.000	0.815	0.462	0.026	0.214	-0.275	0.000	0.754	-0.830
presence of dams	0.000	0.168	0.484	0.000	0.619	-0.787	0.000	0.717	-1.361
current speed	0.000	0.147	-0.069	0.000	0.307	0.232	0.000	0.206	0.330
shade	0.000	0.140	-0.068	0.000	0.245	0.153	0.000	0.072	0.269
silt	0.000	0.005	0.064	0.000	0.904	-0.142	0.000	0.332	-0.247
sand	0.042	0.283	0.018	0.266	0.801	0.019	0.953	0.834	0.001
pH	0.000	0.001	0.271	0.000	0.000	0.304	0.554	0.000	0.039
conductivity	0.032	0.000	0.074	0.000	0.000	-0.378	0.000	0.009	-0.429
chloride	0.000	0.027	0.222	0.000	0.006	-0.651	0.000	0.000	-1.128
ammonium	0.000	0.193	0.071	0.000	0.504	-0.289	0.000	0.116	-0.541
nitrate	0.000	0.765	-0.061	0.000	0.000	0.226	0.000	0.000	0.346
Kjeldahl nitrogen	0.039	0.207	0.046	0.000	0.372	-0.433	0.000	0.085	-0.736
total phosphate	0.748	0.093	0.005	0.000	0.603	-0.237	0.000	0.259	-0.293
oxygen content	0.000	0.633	-0.030	0.000	0.539	0.135	0.000	0.879	0.174

The results were different for the number and fraction of rare taxa (Table 5). In both analyses the estimated regression coefficients were higher than using taxa richness for most of the environmental variables. The same variables had a positive effect on the number and the fraction of rare taxa. Only the variables depth and filamentous algae, showed a positive correlation in one case and a negative correlation in the other, but these variables were not significant.

Table 4. Estimated regression coefficients, standard deviation and t-value for the selected models for taxa richness, number of rare taxa and fraction of rare taxa in streams.

	regression coefficient	standard error	t-value
model I: taxa richness			
Constant	3.118	0.319	9.77
depth	0.2145	0.0338	6.35
width	0.1342	0.0342	3.92
pH	0.1639	0.0375	4.37
conductivity	-0.1313	0.0346	-3.80
total phosphate	-0.0532	0.0181	-2.94
model II: number of rare taxa			
Constant	0.532	0.565	0.94
natural profile	0.8253	0.0928	8.89
pH	0.4344	0.0756	5.75
chloride	-0.4093	0.0837	-4.89
conductivity	-0.3065	0.0630	-4.87
nitrate	0.1793	0.0295	6.08
model III: fraction of rare taxa			
Constant	-1.778	0.576	-3.09
natural profile	0.7981	0.0923	8.65
width	-0.3583	0.0427	-8.38
pH	0.3790	0.0790	4.80
chloride	-0.6673	0.0922	-7.24
conductivity	-0.2179	0.0620	-3.52
ammonium	-0.1512	0.0360	-4.20
nitrate	0.2141	0.0284	7.54

Table 5. Screening results of the environmental variables for taxa richness, number and fraction of rare taxa in channels, including the estimated regression coefficient of the marginal model (beta), the p-valu+e of the marginal test (p-marg) and of the conditional test (p-cond).

	taxa richness			number of rare taxa			fraction of rare taxa		
variable	p-marg	p-cond	beta	p-marg	p-cond	beta	p-marg	p-cond	beta
width	0.005	0.037	-0.053	0.000	0.017	-0.185	0.008	0.090	-0.134
depth	0.011	0.003	0.079	0.578	0.001	0.051	0.760	0.015	-0.028
in natural area	0.003	0.723	0.142	0.000	0.000	1.176	0.000	0.000	1.067
groundwater seepage	0.375	0.019	0.039	0.000	0.158	0.614	0.000	0.008	0.592
water inlet	0.000	0.016	0.174	0.000	0.220	-0.457	0.000	0.056	-0.648
intermittency	0.132	0.337	-0.196	0.221	0.773	0.397	0.068	0.319	0.615
peat	0.000	0.873	0.214	0.000	0.037	0.705	0.000	0.046	0.503
clay	0.000	0.024	-0.185	0.000	0.790	-0.602	0.001	0.515	-0.424
sand	0.981	0.039	0.001	0.000	0.077	0.443	0.000	0.010	0.450
floating vegetation	0.000	0.347	0.031	0.097	0.028	-0.043	0.002	0.011	-0.089
filamentous algae	0.000	0.058	0.070	0.409	0.921	0.029	0.207	0.270	-0.045
emergent vegetation	0.285	0.763	-0.011	0.068	0.137	0.053	0.019	0.188	0.072
submerged vegetation	0.000	0.024	0.061	0.000	0.125	0.098	0.124	0.435	0.042
pH	0.281	0.000	-0.033	0.000	0.702	-0.351	0.000	0.090	-0.375
conductivity	0.000	0.000	-0.247	0.000	0.381	-0.405	0.006	0.297	-0.187
chloride	0.000	0.000	-0.153	0.000	0.629	-0.278	0.002	0.081	-0.173
ammonium	0.000	0.996	-0.105	0.000	0.112	-0.306	0.000	0.066	-0.208
nitrate	0.000	0.321	-0.073	0.000	0.581	-0.167	0.000	0.586	-0.105
total nitrogen	0.000	0.856	-0.192	0.000	0.243	-0.418	0.000	0.260	-0.309
total phosphate	0.000	0.066	-0.139	0.000	0.921	-0.375	0.000	0.479	-0.238

Table 6. Estimated regression coefficients, standard error and t-value for the selected models for taxa richness, number of rare taxa and fraction of rare taxa in channels.

	regression coefficient	standard error	t-value
model I: taxa richness			
constant	3.314	0.287	11.55
depth	0.1043	0.0334	3.12
chloride	-0.1355	0.0255	-5.30
conductivity	-0.1869	0.0265	-7.06
pH	0.2359	0.0405	5.82
total phosphate	-0.0675	0.0227	-2.97
submerged vegetation	0.03322	0.00944	3.52
water inlet	0.1141	0.0429	2.66
model II: number of rare taxa			
constant	0.336	0.134	2.50
total nitrogen	-0.3608	0.0660	-5.47
clay	-0.368	0.139	-2.64
in natural area	0.926	0.129	7.18
model III: fraction of rare taxa			
constant	-4.039	0.166	-24.28
total nitrogen	-0.2046	0.0766	-2.67
floating vegetation	-0.0930	0.0304	-3.05
in natural area	0.896	0.130	6.92
groundwater seepage	0.363	0.128	2.84

For the number of rare taxa only the environmental variables nature function, clay and total nitrogen were included in the best model (Table 6). The fraction of rare taxa was best modeled by including total nitrogen, floating vegetation, nature function, and groundwater seepage.

Taxa richness and number of rare taxa in relation to ecological quality

In general, taxa richness at the selected homogeneous group of channel sites was higher than at the selected stream sites group (Table 7). Taxa richness in relation to ecological quality showed an optimum curve for streams and channels. For streams the sites with good ecological quality had the lowest taxa richness and highest taxa richness was observed at sites with poor ecological quality. Taxa richness in class 2 significantly differed from classes 1 and 4. Differences between the other classes were not significant (Table 7). In channels taxa richness was highest at sites with moderate ecological quality and lowest at sites with bad ecological quality. Taxa richness significantly differed between all ecological quality classes.

Table 7. Mean taxa richness and number of rare taxa (distribution classes uncommon, rare, and very rare) for the ecological quality classes in streams and channels and significant difference between classes of ecological quality.

ecological quality class	number of samples	mean no. of taxa per sample	significant difference with ecological quality class ($p<0.05$)
streams - taxa richness			
1: bad	12	27.0	2
2: poor	50	35.9	1, 4
3: moderate	35	25.2	none
4: good	53	20.9	2
channels - taxa richness			
1: bad	14	26.6	all
2: poor	132	36.8	all
3: moderate	134	67.3	all
4: good	75	57.3	all
streams - no. rare taxa			
1: bad	12	1.0	3, 4
2: poor	50	1.1	3, 4
3: moderate	35	2.6	all
4: good	53	5.2	all
channels - no. rare taxa			
1: bad	14	0.1	3, 4
2: poor	132	0.5	3, 4
3: moderate	134	0.9	all
4: good	75	2.1	all

The mean number of rare taxa was higher in stream samples. The number of rare taxa increased from sites with bad ecological quality to sites with good ecological quality for both streams and channels. Mean numbers of rare taxa were not significantly different between ecological quality classes 1 and 2 in streams and channels (Table 7).

Discussion

About half of the rare taxa occurring in the Netherlands were observed in the streams and channels sampled. Probably, the remaining rare taxa occur in other water types or are difficult to collect. The number of rare taxa in the total data set and the average per sample were higher for streams than for channels. This was true for the complete data set but also for each ecological quality class (Table 7). This means that a difference between the number of unimpacted sites in stream or channel data can not be an explanation. Evolutionary processes might explain this phenomenon. In general, there are groups of taxa that only occur in running waters because streams have been more permanent over the geological time than standing waters, because the latter fill

with deposits and disappear. River fauna therefore has had a better chance to develop and adapt itself to the environmental conditions (Hynes, 1972). This is reflected in our results, not only by the higher number of rare taxa but also because there are more rare taxa that only occur in streams than taxa that only occur in channels (Table 2). Another explanation might be that standing waters, especially small ones are more dynamic, for example, temperature and oxygen content show larger fluctuations. Taxa occurring in such water bodies need to have a high dispersion capacity and a high tolerance for these dynamics. A large part of all Dutch taxa is adapted to a broad range of environmental conditions and is therefore common. These taxa can occur in standing as well as in flowing waters, the latter because there are always habitats in which current velocity is relatively low. Rare taxa are often not adapted to environmental fluctuations and therefore their number is lower in channels.

Within both water types there was variation in taxa richness and the number of rare taxa. In our study, taxa richness was not positively related to environmental variables indicating natural conditions (Tables 3, 4, 5, and 6). But, the initial datasets were large and contained a number of stream and channel types. Habitat variables influenced taxa richness. This is illustrated for example by the fact that the number of taxa in streams was related to width and depth (Table 3 and 4). This was also observed by other researchers. Boon (2000) found that the number of taxa is not always related to naturalness or impairment but also to the water type. Wright *et al.* (1998) confirmed Boons observation in a study of taxa richness for 614 reference sites in which they observed that extremely taxon-poor sites were confined to physically harsh but natural environments. Even within a river the number of taxa can differ. Vannote *et al.* (1980) concluded that taxa richness is dependent on the stream order; taxa richness reaches a maximum in mid-order reaches. This can explain the importance of the variables width and depth in our study. Statzner and Higler (1986) correlated high taxa richness to sites where major changes in stream slope create increased hydraulic variability and microhabitat variability. But, Marchant *et al.* (1999) showed there was no unimodal trend along these gradients, or any other trend.

Habitat variables do obviously influence taxa richness at a site. Therefore, habitat variables were excluded by choosing groups of sites within one stream or channel type but different ecological quality. The relation between taxa richness and ecological quality appeared to be represented by optimum curves for streams and channels (Table 7). There was no linear relationship, which makes the use of taxa richness as a variable in assessment difficult. For channels, differences between all quality classes are significant and bad and poor ecological status have lower taxa richness than moderate and good ecological quality. This provides opportunities for using this variable in assessment. But for streams, using taxa richness is impossible because differences between ecological quality classes are not in all cases significant, e.g., bad and good ecological quality are not significantly different. From the analyses of the number of rare taxa can be concluded that at bad ecological status the community

consists of almost only common taxa, while at good ecological status the number of rare taxa is higher. Thus, taxa richness may be similar, but the taxa composition is probably completely different. This was also shown by clustering and ordination results (Verdonschot and Nijboer, 2004). Our results correspond with the intermediate disturbance hypothesis, which states that at intermediate disturbance taxa richness is highest (Ward and Stanford, 1983). Pianka (1978) related taxa richness to the range of available resources, the niche breadth of the species, niche overlap, and the saturation of niches with more species or more individuals of few species. Combining these two concepts would imply that at intermediate disturbance more resources or niches become available which allows for a higher taxa richness. But other authors found different results. Taxa richness is the most widely used evaluation measure in benthic macroinvertebrate studies of pollution effects (Resh and McElravy, 1993). Resh (1994) and Sandin and Johnson (2000) stated that taxa richness appears to be a good metric in environmental assessment studies. Rapport (1991) demonstrated that ecosystems affected by stress often show reduced taxa richness, with the disappearance of sensitive taxa, a predominance of pollution sensitive taxa and more mono-specific communities. Analysis of metrics, calculated from macroinvertebrate collections from the Fraser River (Resh *et al.* 2000) clearly showed that richness metrics were the most useful of all the types of metrics tested, in terms of ability to indicate impairment when it occurs and not indicating impairment when it does not occur. The difference between this study and earlier work is probably the cause of degradation. In the Dutch streams not pollution by organic or toxic substances but hydromorphological degradation plays the major role. Hydromorphological degradation (straightening and regulation of streams) often results in macrophyte growth, which provides a habitat for standing water species. As long as the stream is not too polluted the common running water species remain, thus taxa richness increases. For channels, the major variable of influence is nutrient enrichment. Nutrient enrichment causes an initial increase in species in naturally oligotrophic systems, which was also shown by Angermeier and Karr (1994).

The number of rare taxa showed different patterns. The number of rare taxa was positively correlated with naturalness and negatively with disturbance variables in both streams and channels (Tables 3, 4, 5, and 6). In streams, the number of rare taxa appeared to be positively related to low nutrient contents, a natural profile, meandering, the absence of dams, and a high current speed. In morphologically disturbed streams, indicated by the presence of dams the number of rare taxa was lower. In channels, the number of rare taxa was positively related to seepage water instead of water inlet (this is water from other areas, for example from the river Rhine that is imported into the polder areas if there is not sufficient water during summer), low nutrient contents, and low conductivity. The latter factors are often related to each other. Many channels are enriched by run-off of nutrients from agricultural land or by the inlet of nutrient and ion rich river water in summer (Roelofs, 1991; Smolders

and Roelofs, 1995). The relationships between naturalness and the number of rare taxa were stronger than for taxa richness. Habitat variables seemed to play a minor role, although width, depth and soil type were related to the number of rare taxa. Vannote *et al.* (1980) also observed a relation between habitat and the number of rare taxa. Headwaters often supported the presence of rare taxa, many of which only occur in these small watercourses. This can also explain the importance of the variables width and depth in our study.

The number of rare taxa showed an increase from bad to good ecological status for streams and channels within one water type (Table 7). This means that rarity is a useful variable in ecological assessment. Because the number of rare taxa decreased if ecological quality decreased the initial increase in taxa richness is due to an increase of common taxa. Differences in numbers of rare taxa between ecological quality classes were significant except for the difference between bad and poor ecological status. The relation between the number of rare taxa and ecological quality can be explained by the fact that those taxa that are rare in the Netherlands are the ones that are related to natural undisturbed waters, because the number of these waters has strongly decreased. For example, only about 4% of the Dutch streams is still natural (Verdonschot and Nijboer, 2002), thus the species inhabiting these waters have become rare. Gaston (1994) also suggested that taxa that were once common might have become rare because their habitats are affected by human activities. In countries with many unimpacted waters, using the same criteria for rarity (Nijboer and Verdonschot, 2004) could result in a selection of rare taxa that are not related to undisturbed sites but to other variables. However, in other countries similar results were found, e.g., Cao *et al.* (1998), who observed more rare taxa at unimpacted sites. Lenat and Resh (2001) observed for coastal plain streams that most slow flowing swamp streams had a low diversity and were dominated by tolerant organisms, but high numbers of rare and unusual taxa characterized sites with the highest water quality (Lenat and Resh, 2001).

Rare taxa can be useful in ecological assessment, although the number of rare taxa in a sample is often quite low and thus difficult to collect. In streams the average number of rare taxa at sites of good ecological status is 5, in channels this is 2. If these numbers of rare taxa are found at a site, it is quite sure that the ecological quality is good, but if no rare taxa are observed the ecological quality does not have to be bad. There always is a chance that a rare species is missed, especially if it is a species that occurs in low abundances. But, this chance is not higher than for common species, because there is no relation between the distribution classes used and abundance of the species as was shown by Nijboer and Schmidt-Kloiber (2004). Our study showed that with the standard sampling procedure used in Europe the number of rare species can be related to ecological quality.

In conclusion, the number of rare taxa is a useful variable in ecological assessment. The present study confirmed that the number of rare taxa increased from

bad to good ecological quality, showing significant differences between ecological quality classes. The threshold value for the number of rare taxa that indicates good ecological status depends on the water type. In channels a lower number of rare taxa were indicative for natural conditions than in streams. Taxa richness showed no clear relation with human impact. For taxa richness habitat variables were more important. But also within a narrow defined water type there was no linear response of taxa richness to ecological quality and differences between ecological quality classes were in many cases not significant. This is due to the impact variables hydromorphological degradation for streams and nutrient enrichment for channels, both one of the major impact variables in Europe. Both variables result in an initial increase in the number of common species before a decrease occurs. Therefore, taxa richness is less applicable in ecological assessment.

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6 Comparison of clustering and ordination methods implemented to the full and partial data of benthic macroinvertebrate communities in streams and channels

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Abstract

We analysed benthic macroinvertebrates data from streams and channels with three techniques: the Self-Organising Map (SOM), non-hierarchical clustering (NHC) and Canonical Correspondence Analysis (CCA). Within the analyses we tried different numbers of clusters (40 for both streams and channels, and 19 for channels and 23 for streams). Furthermore, we repeated the analyses with reduced taxa data. We used indicative taxa, dominant taxa or a selection of 5 taxonomic groups. The SOM and NHC classifications resulted in different distribution of the sites over the clusters. The isolation values for the NHC classification were higher, indicating more distinct clusters. However, the number of sites was unevenly distributed over the clusters. In SOM the sites were more evenly distributed over the clusters. The SOM-classification had a higher number of taxa in each cluster, which means that the overlap in taxa composition between clusters was higher. Probably, the SOM classifies sites together that have comparable abundances for the most dominant taxa and a similar number of taxa, rather than exactly the same species composition.

Reducing the number of clusters resulted in similar isolation values but in different classifications. This makes it difficult to interpret the result of the classification. It is hard to choose the most suited number of clusters for a certain data set.

Reducing the taxa data resulted in different classifications. The differences were larger for SOM than for NHC. Using 5 taxonomic groups resulted in the largest deviation, followed by dominant taxa and indicative taxa, respectively. This means one should be careful in reducing taxa data without comparing the results with the results of analysing the complete data set.

Plotting the environmental variables on the SOM resulted in similar gradients in the environment as resulting from the CCA. Both techniques add information to each other, CCA focuses on the length and direction of gradients and SOM focuses on the distribution over the clusters on the map. Variables that seemed of less importance using CCA could be important for a single cluster, as appeared from the SOM results. Reducing the taxa data had not a direct effect on the relation with environmental variables, only for the variables that were less important in explaining taxa variation in the data.

Introduction

We analysed benthic macroinvertebrates data from streams and from channels with three techniques: the Self-Organising Map (SOM), non-hierarchical clustering (NHC) and Canonical Correspondence Analysis (CCA). These techniques are often used in bioassessment. Assessment of the integrity of biological elements of surface waters is an important aspect in water management (Barbour *et al.*, 2000). To restore surface waters, managers need techniques to identify the present community and to predict which community they can expect if the environment changes either due to degradation or to restoration measures (Verdonschot and Nijboer, 2000). Therefore, understanding community patterns is a fundamental basis for ecosystem management.

Benthic macroinvertebrates are recognised as one of the most reliable biological indicator groups in aquatic ecosystems (Hellawell, 1986). They play a key role in food web dynamics, linking producers and top carnivores, and a number of species have clear responses to environmental variables. Their spatial sedentariness and intermediate life span, from several months to several years, make macroinvertebrates ideal as for an integrative and continuous indicator group of water quality (e.g., Sládeček, 1979; Hellawell, 1986).

Many useful biological indices use benthic macroinvertebrates (reviews of biological indices and metrics: Metcalfe, 1989; Resh and Jackson, 1993; Verdonschot, 2000). For a long time, biological assessment of water quality had been uni-dimensional (Cairns and Prett, 1993) and focussed, for example, on organic pollution. However, Karr (1991) stressed that species can react to a complex of factors and that they can also influence each other, for example by competition, thus the use of one species as indicator has its shortcomings. During the last decades, ecological assessment systems have been developed. This was stimulated by the development of integrated ecological indices (Karr *et al.*, 1986; EPA, 1988). In ecological assessment the overall environment is added to the biological component (Odum, 1971), and the combination of species composition and environmental variables is used to assess the quality of surface waters (Verdonschot, 1990). In these ecological assessment systems often a group of organisms, or even a whole community is used as bio-indicator e.g.,

in RIVPACS (Wright *et al.*, 1993; Wright, 2000) and EKOO (Verdonschot and Nijboer, 2000). Multivariate techniques such as principal component analysis, cluster analysis, and correspondence analysis have been used to understand these ecological data, to extract communities, and to relate these to the environment (e.g., Gauch, 1982; Jongman *et al.*, 1987; Ludwig and Reynolds, 1988; Legendre and Legendre, 1998). Assessment systems, such as EKOO and RIVPACS, are based on a stepwise progression of clustering and ordination. The basic unit is the community, which is interpreted using cluster analysis.

Recently, artificial neural networks (ANN) have been used for classifying groups (e.g., Chon *et al.*, 1996) and patterning relationships between variables (Lek *et al.*, 1996). The ANN appeared to be a versatile tool for dealing with problems to extract information out of complex and non-linear data (Hoang *et al.*, 2001), and could be effectively applied in classification and association (Lek and Guegan, 2000). ANNs have been successfully applied to classify communities and to predict species distribution, communities and community variables, such as diversity (Lek and Guegan, 2000; Recknagel, 2002). Others have related community characteristics to environmental variables (e.g., Lek *et al.*, 1996; Recknagel *et al.*, 1997). Among ANN techniques, the self-organising map (SOM), which is based on an unsupervised learning algorithm, is often used to analyse the community structure. In several applications, e.g., Chon *et al.* (1996, 2000) and Park *et al.* (2001, 2003), SOM was successfully used to pattern benthic macroinvertebrate communities.

However, analysing community patterns is difficult because the data sets are non-linear and composed of many species varying over different locations and time (Chon *et al.*, 2000) and with a different distribution and density. At a site, only part of the present community is collected at a certain moment. Therefore, each sample contains some information about the community but none is complete. Analysing large data sets is always an interpretation of the real situation; it is difficult to make the community structure apparent (Giraudel and Lek, 2001).

In classification, sampling sites are clustered to reduce the variability and complexity of ecosystems and to make the results more useful in water management. Techniques can give insight in the structure of communities, but the results can differ between techniques or within a technique depending on the choices that are made, e.g., the number of clusters and the basic algorithm. If the data set is small and shows clear gradients, different techniques show similar results (Giraudel and Lek, 2001). But if the gradients in the data are less clear, different techniques might result in different community structures.

Furthermore, classification and ordination results could differ if some species are excluded from analysis. This has been frequently conducted because rare species can add noise to the analyses (Gauch, 1982; Marchant, 2002) or because processing complete macroinvertebrate samples implies high costs. For management purposes, it would be more effective if the number of species that is necessary as input in an

assessment system could be reduced. Above that, results of clustering data sets with over 500 species are hard to analyse and to interpret. Many researchers reduced the species data before using ANN to pattern the community. Chon *et al.* (2000) for example, summed the densities of the taxa to seven selected taxa. Park *et al.* (2003) expressed the biotic data as EPTC (Ephemeroptera, Plecoptera, Trichoptera, and Coleoptera) richness. Hoang *et al.* (2001) used only presence/absence of the 37 most common taxa. Walley and Fontama (1998) used the BMWP score or number of families as a biological index. However, these researchers did not compare the results of analysing only part of the data with analysing the complete data set.

The aims of our study were: (1) to investigate the differences between classification with an unsupervised artificial neural network and with a classical clustering technique using two large data sets, (2) to test the stability of the classification results from both techniques, if only parts of the taxa data are used, (3) to compare the gradients in environmental variables resulting from an artificial neural network with those resulting from a classical ordination technique, and (4) to study the ordination results using the complete and reduced data sets.

Materials and methods

Data

Results of comparing techniques could be different between data sets, depending on the total number of sites or taxa, the number of taxa per site, the distribution of the species over the sites or the distribution of the individuals over the taxa. Therefore, two different data sets, both including samples taken in different seasons were used in this study. The first data set contained 563 samples from streams and included 767 macroinvertebrate taxa. The second data set existed of 408 samples from small channels, less than 15 m wide, used for drainage in agricultural areas. In this data set 695 macroinvertebrate taxa were present. The benthic macroinvertebrate data in both data sets were collected by water district managers all over the Netherlands by using a standard sampling protocol (Verdonschot, 1990). In summary, a 5 m surface sample was taken with a pond dip net. The sample was divided over the dominant habitats representative for the stream stretch or the channel. In both data sets animals were identified to species level where possible. The abundances were log-transformed ($\ln(x+1)$) to normalise the data distribution.

Water district managers measured environmental variables. The variables measured and the methods differed between water managers. Therefore, in a first step, variables that were measured in less than 90% of the samples were removed from the data. In a second step, environmental variables were selected from the total data set based on their importance, which appeared from the first ordination results and expert opinion. Different variables were selected for either streams or channels.

For all variables annual means were used. To estimate lacking values for environmental variables (less than 10% of the sites per variable) average values were used from samples that were classified in the same cluster using non-hierarchical clustering (paragraph 2.3). Finally, the stream data included 19 environmental variables, the channel data 22 variables.

The Self Organising Map (SOM)

We used the SOM to pattern and classify species communities and relate them to environmental variables. The SOM is an unsupervised learning algorithm for clustering, visualisation, and abstraction. The SOM is used to represent the data set in another, more usable form; it is an approximation to the probability density function of the input data (Kohonen, 2001). The SOM consists of two different units (i.e. computational units) of input and output layers, connected by the computational weight vectors (i.e. connection intensities). To train the SOM, initially the community data with species density were subjected to the SOM as input. When the input vector x is sent through the network, each neuron k of the network computes the summed distance between weight vector w and input vector x. The output layer consists of N output neurons which usually constitute a two dimensional grid because of better visualisation. The form of the output layer is a hexagonal lattice, because it does not favour horizontal and vertical directions as much as the rectangular array (Kohonen, 2001). Among all output neurons, the best matching unit (BMU) which has minimum distance between weight and input vectors becomes winner. For the BMU and its neighbourhood neurons, the new weight vectors (w) are updated by the SOM learning rule. The training is usually done in two phases: first rough training for ordering with a large neighbourhood radius, and then fine tuning with a small radius. This results in training the network to classify the input vectors by the weight vectors they are closest to. The detailed algorithm of the SOM is described by Kohonen (1989, 2001) together with theoretical considerations, and Chon *et al.* (1996) and Park *et al.* (2003) included ecological applications.

The number of output neurons (map size) affects the resolution of patterns resulted from the SOM. Therefore, the map size is an important parameter. To find the optimum map size, we trained the SOM with different map sizes ranging from 15 to 200 output units. Finally, we chose 40 (i.e., 8×5) as number of SOM output neurons on the 2D hexagonal lattice based on our experience and reasonable ecological meaning with each technique. The learning process of the SOM was carried out using the Matlab SOM Tool Box (Alhoniemi *et al.*, 1999; The MathWorks, 2001).

After training the SOM, hierarchical cluster analysis using Ward's linkage method was conducted to find clusters on the units of the SOM map according to their similarities.

Characterisations of the clusters were made using expert judgement and literature about the indicative taxa (Mol, 1984; Gittenberger *et al.*, 1998; Drost *et al.*, 1992; Geijskes and Van Tol, 1983; Bos and Wasscher, 1997; Smit and Van der Hammen, 2000).

To analyse the relationship between biological and environmental variables, the mean values of environmental variables were visualised on the SOM map. To do this, we calculated the mean value of each environmental variable in each output unit of the trained SOM (Park *et al.*, 2003) and represented these means by a grey scale.

Non-hierarchical clustering (NHC)

The same data sets were clustered by means of the NHC using the program FLEXCLUS (Van Tongeren, 1986). The strategy is based on an initial, non-hierarchical clustering, following the algorithm of Sørensen (1948) for a site-by-site matrix based on the similarity ratio, using species abundances. During this initial clustering, sites are fused according to single linkage but a fusion is skipped when two sites with a lower resemblance to each other than a specified threshold would become members of the same cluster. The value of the threshold depends on the number of sites clustered and the cluster homogeneity. The homogeneity of a cluster is defined as the average resemblance (based on the similarity ratio) of the sites of this cluster to its centroid. The initial clustering is optimised by relocative centroid sorting. Large and/or heterogeneous clusters are divided, small and/or comparable clusters (with a high resemblance) are fused, and then sites are relocated. During the relocation procedure, each site is compared to each cluster (as it was before relocation of any site) and, if necessary, moved to the cluster to which its resemblance is highest. Before a site is compared to its own cluster, the respective site is removed from that cluster and the new cluster centroid is computed.

To make both techniques (SOM and NHC) comparable the number of resulting clusters should be the same. In the SOM modelling procedure 40 output units were chosen which were later grouped into 19 groups for streams and 23 groups for channels. The same numbers of clusters were chosen in FLEXCLUS. Therefore, some runs were done to explore the correct threshold value to obtain this number of clusters. After the initial clustering 50 relocation cycles were carried out. This was sufficient to result in stable clusters.

Comparison of SOM with NHC classifications

Distribution of the sites over the clusters

First, the distributions of the sites over the SOM and the NHC clusters were compared. This was done by constructing a matrix, with clusters of SOM in the rows and clusters of NHC in the columns. In the matrix cells the number of sites that

occurred in the respective combination of SOM and NHC clusters was given. For each row and column the total number of sites and the maximum number of sites were calculated. The total number of sites minus the maximum number of sites was considered as the deviation. The total deviation was calculated for all rows together as well as all columns together. Finally, the percentage was calculated by dividing the total deviation by the total number of sites. Two types of error were calculated, (1) type a error, calculated over the rows, in this case the percentage of sites that were combined in a cluster in the SOM but spread over other clusters using NHC, and (2) type b error, calculated over the columns, in this case the percentage of sites that were combined in a cluster in NHC but spread over other clusters in the SOM.

Cluster characteristics

The stability of a classification can be expressed by the mean isolation value over all clusters. The mean isolation value is calculated for each cluster by dividing the homogeneity of a cluster by the resemblance of a cluster to the most similar cluster. The homogeneity is the average similarity between all combinations of two sites in the cluster. The resemblance is the similarity of the sites within one cluster to the sites of the most similar cluster. If the isolation is higher than 1, the homogeneity of the cluster is higher than the resemblance to the most similar cluster. In these calculations the similarity measure used is the similarity ratio.

For all NHC classifications minimum, average, and maximum homogeneity, resemblance and isolation were calculated. For the resulting site groups from the SOM the same values were calculated using the FLEXCLUS program. Therefore, the classification resulting from the SOM modelling was introduced as a fixed classification into FLEXCLUS.

An additional cluster characteristic is the distribution of the sites over the clusters. We used the number of clusters with only one site and the maximum number of sites within a cluster.

Typifying taxa

For each cluster a set of indicators was established by using a calculation of typifying weights. A typifying weight represents the indicative value of a species for a cluster. The typifying weight for a single taxon differs between clusters. The weights for all species per cenotype were calculated using the program NODES (Verdonschot, 1990). The clusters and the sites with the abundances of the taxa were used as input. In NODES the typifying weight of a taxon was calculated per cluster by combining the formulae of constancy, fidelity, and concentration of abundance (Boesch, 1977; Verdonschot, 1984). The higher the weight, the more characteristic the taxon is for a cluster. For example, if a species occurs within one cluster with a high frequency of occurrence and high abundance and it does not occur in any of the other clusters the

typifying weight of the taxon for that cluster is extremely high and low for all other clusters. If a taxon occurs in all clusters in about the same frequency and with similar abundances, the typifying weight of that taxon is low for all clusters. The weights vary from one to twelve (Verdonschot, 1990). The taxa can be divided into four indicator groups: indifferent taxa (weights 1-3), lowly typifying taxa (weights 4-6), moderately typifying taxa (weights 7-9), and highly typifying taxa (weights 10-12).

To compare the results of classification between SOM and NHC, the number of highly typifying taxa (all taxa that have a typifying weight > 10 for one or more clusters) was calculated for classifications with 40 and 19/23 clusters (19 clusters for stream data, 23 clusters for channel data), respectively. The overlap of highly indicative taxa between SOM and NHC classification was further determined by calculating the mean abundance and frequency of occurrence for three groups of taxa: (1) only typifying in SOM, (2) only typifying in NHC, and (3) typifying in both classifications, using stream data and 40 clusters.

Classification with reduced taxa data

We used three methods for taxa reduction. First, we considered the frequency of occurrence and abundance of taxa in the site samples. We excluded the species that occurred at less than 1% of the sites and had an average abundance less than 8. The species that remained in the data were called 'dominant species'.

Second, we considered taxonomic groups according to ecological indicative value. We used species' abundances of five taxonomic groups for streams as well as for channels. These groups include a variety of species, which we thought to be ecologically indicative for the respective water types. For streams we included Ephemeroptera, Chironomidae, Gastropoda, Oligochaeta, and Crustacea. For channels we included Chironomidae, Coleoptera, Crustacea, Heteroptera, and Gastropoda.

The third method of taxa reduction was done after the first SOM including all taxa. We selected indicator taxa (in this study defined as taxa that are indicative for a certain SOM unit). After training the SOM, we considered the weights of the SOM as occurrence probability ranged between 0 and 100% in each SOM unit. In the weight matrix, high values represent a high probability of occurrence in the concerning neuron, while low values represent a low probability of occurrence. For example, a species with maximum 100% in a certain unit displayed, we can observe this species with relatively high abundances in most of the sampling sites assigned in the unit. Using these values we selected species, that had a maximum probability > 5% (in one or more of the output units the probability is > 5%). We considered these selected species as 'indicator species' in this study.

The reduction of species resulted in three new data sets which included about 28-56% of the species that were included in the complete data sets (Table 1). With the

smaller data sets SOM and NHC were repeated, using the same methods. Results of the complete data sets were compared with the results using part of the data by comparing (1) site distribution over the clusters and (2) measures for classification consistency.

Table 1. The number of taxa in each of the data sets. The numbers in parenthesis are the percentages of the number of taxa in the complete data set.

water type	number of species in complete data set	indicator taxa	dominant taxa	5 taxonomic groups
streams	767 (100)	270 (35.2)	214 (27.9)	255 (33.2)
channels	695 (100)	310 (44.6)	241 (34.7)	391 (56.3)

Comparison of SOM with Canonical Correspondence Analysis (CCA)

The results of the SOM were compared with ordination results to study the role of the environmental variables in the data. Within SOM the most important environmental variables were selected using the distribution of variables on the map. Used in this way, the SOM is a method for relating community data to environmental data. The state of the art method to this aim is Canonical Correspondence Analysis (CCA) (Ter Braak and Verdonschot, 1995). This is the reason why we compared the results of the SOM with CCA. CCA was carried out with the same data using the program CANOCO (Ter Braak and Šmilauer, 2002). Before analysis, data were transformed. Species data were transformed into Preston classes (Preston, 1962; Verdonschot, 1990) and environmental variables except for pH were log transformed (ln(x+1)).

Ordination with reduced taxa data

Ordination results were compared between using the complete data set, the data with indicator taxa, with dominant taxa and with five taxonomic groups. To judge the strength of the ordination, the eigenvalues of the first four axes were used. Both measures illustrate the variance in the data that is explained by the ordination axes. The first measure includes all variation, the second one only the variation that is explained by the environmental variables.

Forward selection was used to compare the order of importance of the resulting environmental variables between the results using the different data sets. The conditional effects of the variables were used. This means that first the most important variable was chosen, followed by the second variable that explains most of the remaining variation, and so on.

Results and discussion

Patterning sites using the SOM

Channels

Using the data matrix of channels to train the SOM of 40 output units (a 5x8 map), we obtained the distribution pattern of the channel communities. Clustering the 40 units of the SOM with the help of the dendrogram with the Ward's algorithm resulted in 23 clusters, which are illustrated in Fig. 1.

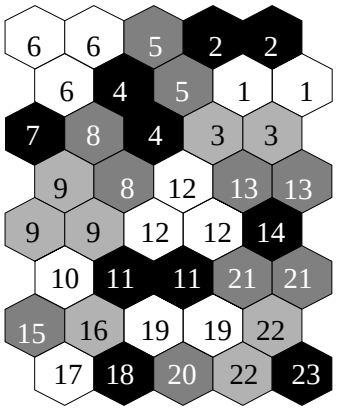

Fig. 1. Classification of the SOM map for channels. Cells with the same number belong to the same cluster. The numbers of these clusters refer to Table 2.

After training the SOM with community data, the mean values of the environmental variables were visualised on the trained SOM map. This technique is useful to identify the associations between environmental variables and communities. On the SOM map, a clear gradient in the distribution of a variable represents a high contribution to the classification (Fig. 2). The results of the SOM for channel data show that some variables have a very restricted distribution over the map (only few cells are darkly coloured) while others had more similar values all over the cells of the SOM (these variables are lightly coloured in many cells). In the right upper part of the SOM map the brackish channels occur. These channels have high chloride levels and a high conductivity. Most of these channels are situated along the coast and their soil mainly consists of clay. In these channels emergent vegetation is dominant. Most of the channels that are wide and deep also occur in the right upper part of the SOM map.

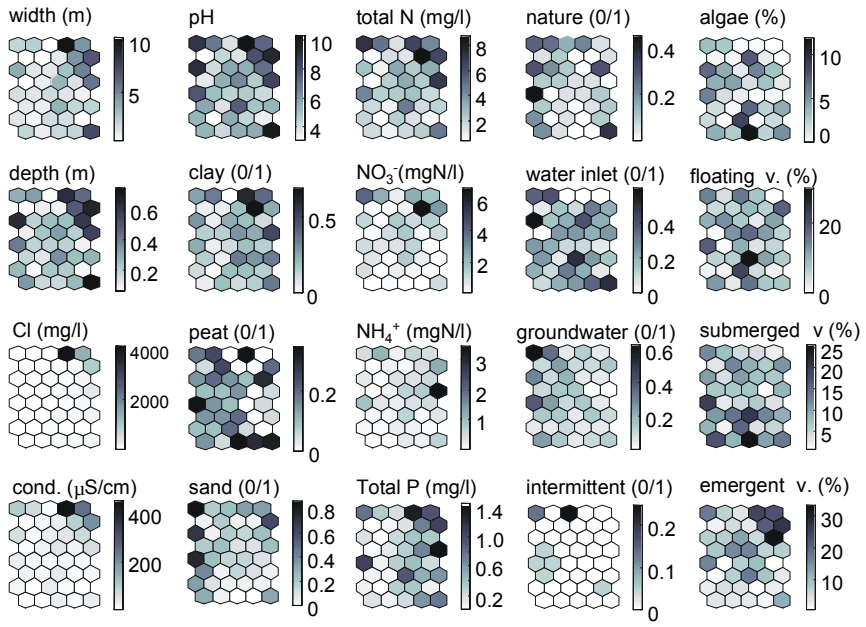

Fig. 2. The distribution of environmental variables over the SOM map for channels, the level of the variables is indicated by a grey scale, ranging from light grey for low values to black for the highest values. (cond.=specific conductivity, v.=vegetation cover).

Eutrophication appeared to be the most important degradation factor. The nutrient levels (TN, TP, NO_3^- and NH_4^+) are high in the upper and right part of the map. In the left and lower part of the SOM map, nutrient concentrations are lower. The channels that are situated in nature reserves seem to have a soil mostly consisting of sand, but natural channels on peat did also occur. Clay channels were in most cases influenced by high nutrient or chloride levels. The vegetation types did not show a clear pattern except for the emergent vegetation that was correlated with the brackish channels. Submerged vegetation had highest % coverage in the lower part of the map, but floating vegetation and algae did not show any gradient. Intermittent channels were characteristic for the middle unit in the upper line in which very shallow channels occur.

For some groups the indications given by the characteristic taxa (Table 2) were clearly linked to the extent of the values for the related environmental variables (Fig. 2). For example, the brackish channels (high chloride level and high specific conductivity) were inhabited by typical brackish water species. The group with submerged vegetation had species that live in vegetation. The left upper group was characterised by Tubificidae, indicating organic pollution, which corresponds with the distribution of the environmental variables. However, differences between groups in the middle of the map and differences between neighbouring groups were often less

clear and only small. These are the channels that have no extreme characteristics and a more overlapping species composition. The indicator species are common species that can occur in many channel types and are not indicative for a specific environment. Some species indicated slowly flowing water, e.g., in group 9 and 5, but this variable was not included in the channel data and therefore not illustrated on the SOM map (Fig. 2).

Table 2. Characterisation of the communities in the 23 channel groups identified by the SOM.

group	characteristic taxa	characterisation
1	*Chironomus* sp.*, Gammarus duebeni, Sigara lateralis*	slightly brackish
2	*Gammarus zaddachi, Palaemonetes varians, Nereis diversicolor*	brackish
3	*Dytiscus circumcinctus, Culex* sp.*, Lestes viridis*	temporary
4	*Ilyodrilus templetoni, Dero digitata, Spirosperma ferox*	sand, no vegetation
5	*Limnodrilus claparedeianus, Macropelopia* sp.*, Gammarus roeselii*	organic soil, slowly flowing water
6	Tubificidae juvenile with hair chaetae, Tubificidae juvenile without hair chaetae, *Psectrotanypus varius*	saprobic
7	*Limnephilus lunatus, Arrenurus virens, Zavrelimyia* sp.	dense vegetation
8	*Arrenurus securiformis, Gyrinus marinus, Ablabesmyia monilis*	peat
9	*Pisidium* sp. *Clanotanypus nervosus, Caenis horaria*	sand, slowly flowing water
10	*Sialis lutaria, Tanytarsus* sp.*, Polypedilum nubeculosum*	vegetation, organic soil
11	*Sphaerium corneum, Athripsodes aterrimus, Hygrotus versicolor*	vegetation, oxygen rich
12	*Triaenodes bicolor, Holocentropus picicornis, Limnesia maculata*	vegetation, oxygen rich
13	*Piscicola geometra, Rhantus frontalis, Cricotopus* gr. *intersectus*	wide
14	*Unionicola crassipes, Hydrovatus cuspidatus, Ablabesmyia longistyla*	wide, eutrophic
15	*Planobarius corneus, Musculium lacustre, Haliplus heydeni*	hypertrophic, filamentous algae, vegetation
16	*Polycelis* sp.*, Xenopelopia* sp*, Laccophilus hyalinus*	wide with organic soil
17	*Asellus aquaticus, Bithynia tentaculata, Planorbis planorbis*	eutrophied, low oxygen level
18	*Stagnicola palustris,* Ceratopogonidae*, Lymnaea stagnalis*	oxygen poor, covered by *Lemna* sp.
19	*Gyraulus albus, Arrenurus crassicaudatus, Haliplus immaculatus*	vegetation, low oxygen level
20	*Arrenurus globator, Haliplus* sp.*, Ilyocoris cimicoides*	vegetation, nutrient rich, organic pollution
21	*Ischnura elegans, Arrenurus latus, Arrenurus sinuator*	vegetation, moderately polluted
22	*Theromyzon tessulatum, Glossiphonia heteroclita, Noterus clavicornis*	large channels, moderately polluted, open, few vegetation
23	*Cricotopus* gr. *sylvestris, Radix peregra, Sigara striata*	hypertrophic channels

Streams

Using the data matrix of streams to train the SOM of 40 output units, i.e. 5x8 map, we obtained the distribution pattern of the stream communities. Clustering the 40 units of the SOM with the help of the dendrogram with the Ward's algorithm resulted in 19 clusters, which are illustrated in Fig. 3.

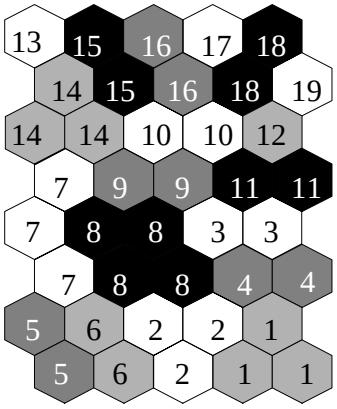

Fig. 3. Classification of the SOM map for streams. Cells with the same number belong to the same cluster. The numbers of these clusters refer to Table 3.

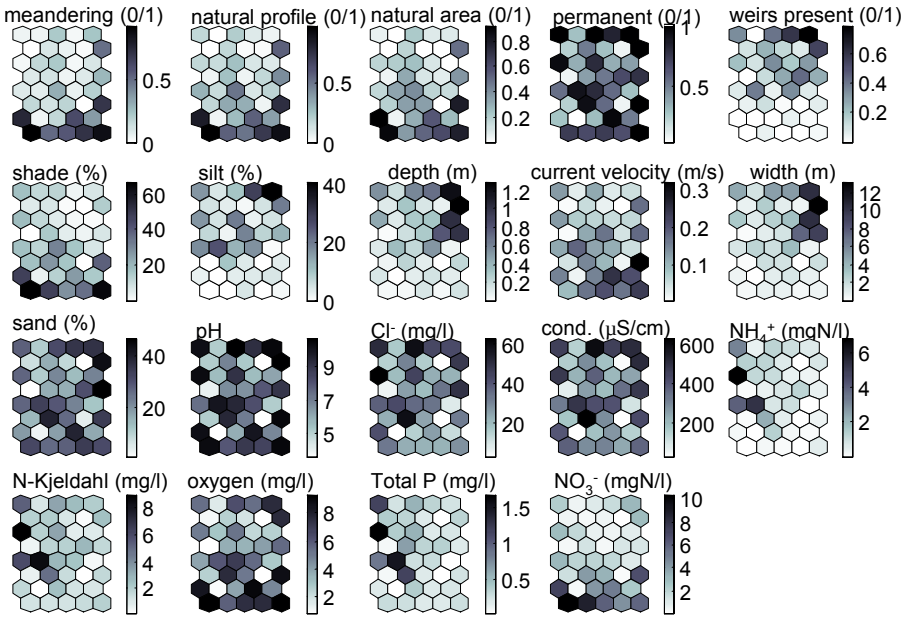

Fig. 4. The distribution of environmental variables over the SOM for streams, the level of the variables is indicated by a grey scale, ranging from light grey for low values to black for the highest values (cond.=specific conductivity).

When mean values of the environmental variables were visualised on the SOM map trained with community data of streams, three gradients appeared to be important (Fig. 4), a dimensional gradient, a nutrient/organic pollution gradient and a morphological alteration gradient. Wide and deep streams are situated in the right

upper part of the SOM map, small shallow streams at the lower and left part of the map. Many of the smaller streams were still in a natural state, meandering, having a natural profile and being situated in nature reserves (often forests). On the contrary, streams in the upper part of the SOM map were influenced by human impact, they were normalised and canalised.

The deeper and wider streams often contained weirs, current velocity was low and silt had deposited. Organic pollution played a role in the left part of the SOM map (N-Kjeldahl, total phosphorus (TP), and NH_4^+ contents were high). The nitrate (NO_3^-) concentration was high in the lower units of the map. The oxygen content was high in the smaller streams with high current velocities.

Table 3. Characterisation of the communities in the 19 stream groups identified by the SOM.

group	characteristic species	characterisation
1	*Gammarus fossarum, Baetis vernus, Hydropsyche angustipennis*	upper-middle course, fast flowing
2	*Gammarus pulex, Sericostoma personatum, Velia caprai*	undisturbed small upper course
3	*Anabolia nervosa, Gammarus roeselii, Mystacides nigra*	normalised lower course, with vegetation
4	*Hygrobates nigromaculatus, Platambus maculatus, Lebertia inaequalis*	undisturbed upper course
5	*Nemoura cinerea, Glyphotaelius pellucidus, Zavrelimyia* sp.	intermittent small upper course
6	*Elodes minuta, Plectrocnemia conspersa, Brillia modesta*	springbrook
7	*Micropsectra* sp.*, Conchapelopia* sp.*, Prodiamesa olivacea*	undisturbed, slowly flowing small upper course
8	Tubificidae juvenile with hair chaetae, Tubificidae juvenile without hair chaetae, *Limnodrilus hoffmeisteri*	stream with organic pollution
9	*Limnodrilus claparedeianus, Cryptochironomus* sp.*, Neumania deltoides*	normalised, slowly flowing middle-lower course
10	*Stylaria lacustris, Limnesia koenikei, Armiger crista*	normalised upper course
11	*Micronecta* sp.*, Caenis luctuosa, Cyrnus trimaculatus*	normalised middle course
12	*Mideopsis orbicularis, Caenis horaria, Ischnura elegans*	sand with silt, slowly flowing
13	*Procladius* sp.*, Pisidium* sp.*, Cricotopus* gr. *sylvestris*	slowly flowing hypertrophic upper-middle course
14	*Chironomus* sp.*, Psectrotanypus varians, Radix ovata*	polluted slowly flowing stream with vegetation
15	*Asellus aquaticus, Helobdella stagnalis, Cloeon dipterum*	normalised lower course
16	*Anisus vortex, Bithynia tentaculata, Sigara striata*	slowly flowing, polluted, low oxygen level
17	*Laccophilus hyalinus, Hygrotus versicolor, Bithynia leachi*	slowly flowing, polluted, moderate oxygen level
18	*Arrenurus globator, Haliplus immaculatus, Limnesia undulata*	middle-lower course, vegetation, very low current velocity
19	*Arrenurus crassicaudatus, Limnesia maculata, Molanna angustata*	normalised middle-lower course with vegetation

The ecological indications of the characteristic taxa mainly confirmed the distribution of the environmental variables over the SOM for streams (Table 3). In the lower part of the map the species of fast flowing upper courses occurred and in the

upper part species that indicate normalised and often larger streams were found. However, sometimes the species indicated a different environment than could be interpreted from the SOM. For example, the species of group 9 and 10 indicate middle-lower courses and upper courses, respectively. From the map appears that the dimensions should be the other way around. Probably, other variables played a role, such as normalisation. Another example is group 16, which seems to be polluted, considering the indicator species. This is not shown by the nutrient concentrations on the SOM, only chloride concentration and conductivity are high. Variables, such as the presence of vegetation, are indicated by the species but were not included in the data analyses. This sometimes explains the differences between interpretation from the species and interpretation from the environmental variables.

Comparison between SOM and NHC classifications

Distribution of the sites over the clusters

The distribution of the sites over the clusters strongly differed between the SOM and the FLEXLCUS classification with the same number of clusters (Table 4). About half of the sites were put in different clusters. This goes for the classification in 40 clusters as well as for the classification in 19/23 clusters (23 channel clusters and 19 stream clusters found on the SOM map). This result was not found by Aguilera *et al.* (2001) who compared the Kohonen Neural Network classification of coastal waters in four groups with numerical classification. Probably the small number of groups and the fact that both of their classifications were based on Euclidean distance caused that the results were quite similar. Chon *et al.* (1996) observed that classification with the Kohonen Network and classification with clustering based on average linkage between groups (Norusis, 1986), showed similar results. However, this was done for a small very distinct data set with 10 sampling sites and 8 tree species. Apparently, if larger complex data sets are used, the results of different techniques are less similar.

Table 4. Comparison of the sites distribution over the clusters using SOM and NHC.

	Streams 40 clusters (% of sites)	Channels 40 clusters (% of sites)	Streams 19 clusters (% of sites)	Channels 23 clusters (% of sites)
type a deviation*	43	44	37	48
type b deviation**	52	53	48	53

* Type a deviation = % of sites that was in one group using SOM, but put in different groups using NHC.

** Type b deviation = % of sites that originates from different groups using SOM and is included in the same group using NHC.

Cluster characteristics

Using the SOM, a less uneven distribution of the sites over the 40 clusters was the result (the maximum number of sites in a cluster was 45 for streams and 23 for channels, Table 5). The classification in 40 clusters resulting from NHC had a much higher maximum number of sites within a cluster, 71 and 79 sites for streams and channels, respectively. Using SOM there were no clusters composed of a single site. Using NHC there were 10 clusters consisting of one site for streams as well as for channels. Classification in 19/23 clusters resulted in higher maximum numbers of sites within the clusters. Again, using SOM the sites were more evenly distributed over the clusters.

Table 5. Cluster characteristics for the SOM classification and the NHC classification (all calculations were done excluding clusters of 1 site).

water type	technique	no.	homogeneity			resemblance			isolation			no. sites	
		clusters	min	mean	max	min	mean	max	min	mean	max	min	max
streams	NHC	40	0.41	0.50	0.66	0.27	0.52	0.76	0.70	1.03	2.41	1	71
streams	SOM	40	0.22	0.42	0.60	0.51	0.69	0.87	0.32	0.61	0.93	2	45
streams	NHC	19	0.32	0.47	0.60	0.27	0.46	0.57	0.73	1.07	1.98	1	100
streams	SOM	19	0.25	0.44	0.58	0.57	0.71	0.87	0.41	0.61	0.83	8	71
channels	NHC	40	0.39	0.53	0.63	0.36	0.53	0.72	0.70	1.01	1.59	1	79
channels	SOM	40	0.25	0.46	0.58	0.38	0.70	0.82	0.46	0.66	1.41	3	23
channels	NHC	23	0.39	0.52	0.63	0.29	0.52	0.69	0.71	1.03	1.58	1	96
channels	SOM	23	0.30	0.46	0.58	0.41	0.73	0.86	0.48	0.64	1.23	3	39

Isolation values were similar for stream and channel data. Both, using 40 or 19/23 clusters, minimum, maximum, and mean isolation were lower for the SOM classification compared to NHC classification. This was due to lower homogeneity and higher resemblance for the SOM classification. Thus, NHC resulted in a classification of more distinct clusters. This could partly be explained by the fact that there were less clusters using NHC if the single sites were not included and that one or two very large clusters were made. To optimise the comparison, the classification in 40 clusters using NHC was repeated for streams, hereby deleting the 10 single sites from the data set. Again 'single site clusters' were formed. The isolation values for the complete classification were similar. This confirmed that the excluded sites were no outliers, otherwise the isolation value would have improved. Apparently, NHC always finds sites that are more different from all the others than the differences between groups of sites. Another explanation for the higher isolation values for the NHC is that the isolation is calculated using the similarity ratio which is also used to cluster the sites with this technique. It is therefore recommended to develop a evaluation measure for the SOM and than test SOM and NHC with this measure.

The average number of taxa per sample and the total number of taxa in the clusters were higher in the SOM classification compared to the NHC classification (Fig. 5a and b) using 19/23 as well as 40 (results not shown) clusters. In NHC, the 'single site clusters' had very low numbers of taxa, which was probably the reason for the separation of these sites. The SOM classification shows a steeper line for the average number of taxa per sample, which means that this technique uses taxa richness to cluster the sites more than NHC does. This can explain the many successful applications of SOM in combination with multilayer perceptron with backpropagation algorithm for the prediction of taxa richness and diversity (e.g., Park *et al.*, 2003). The fact that the SOM clusters contained larger numbers of taxa could be an explanation for the lower isolation values compared to NHC results. If sites are not clustered because they have small group of overlapping taxa but on other features such as taxa richness, which is probably the case for SOM, the clusters will be more heterogeneous and resemble other clusters more, concerning the taxonomic composition.

In four stream clusters and in three channel clusters the number of species was higher in the NHC classification compared to the SOM classification. These were the clusters with a high number of sites. Because the other clusters had fewer sites than most of the SOM clusters, the number of species in the other clusters was lower. Again, the distribution of the numbers of sites over the clusters seems to be important in evaluation of cluster characteristics. Apparently, NHC made one or two large clusters, which included sites with high numbers of taxa. The remaining clusters were small and consisted of samples in which the taxa that occurred in the samples in the large clusters were lacking.

Using 40 clusters the pattern was the same, only the differences between NHC and SOM were slightly smaller. Using NHC the average number of taxa per cluster was 125 for channels and 127 for streams. With SOM the average values were 171 and 168 for channels and streams, respectively. In both classifications the number of taxa had increased by using only 19/23 groups. This indicates that sites that included partly different taxa were put together. This is possible if a number of dominant taxa have high densities and therefore the similarity between the clusters is high.

Fig. 5a also shows that the average number of taxa in the samples in both classifications is higher in the channel data compared to the stream data, although the total number of taxa in the clusters was less for channel data (Fig. 5b) and the overall number of taxa was less in channels (695 taxa) than in streams (767 taxa). This indicates that many taxa in channels are more widespread. In streams, taxa are more restricted to certain sites, which results in a lower number of taxa in the samples, but a higher total number of taxa. However, this was not reflected in higher isolation values for the stream clusters (Table 5).

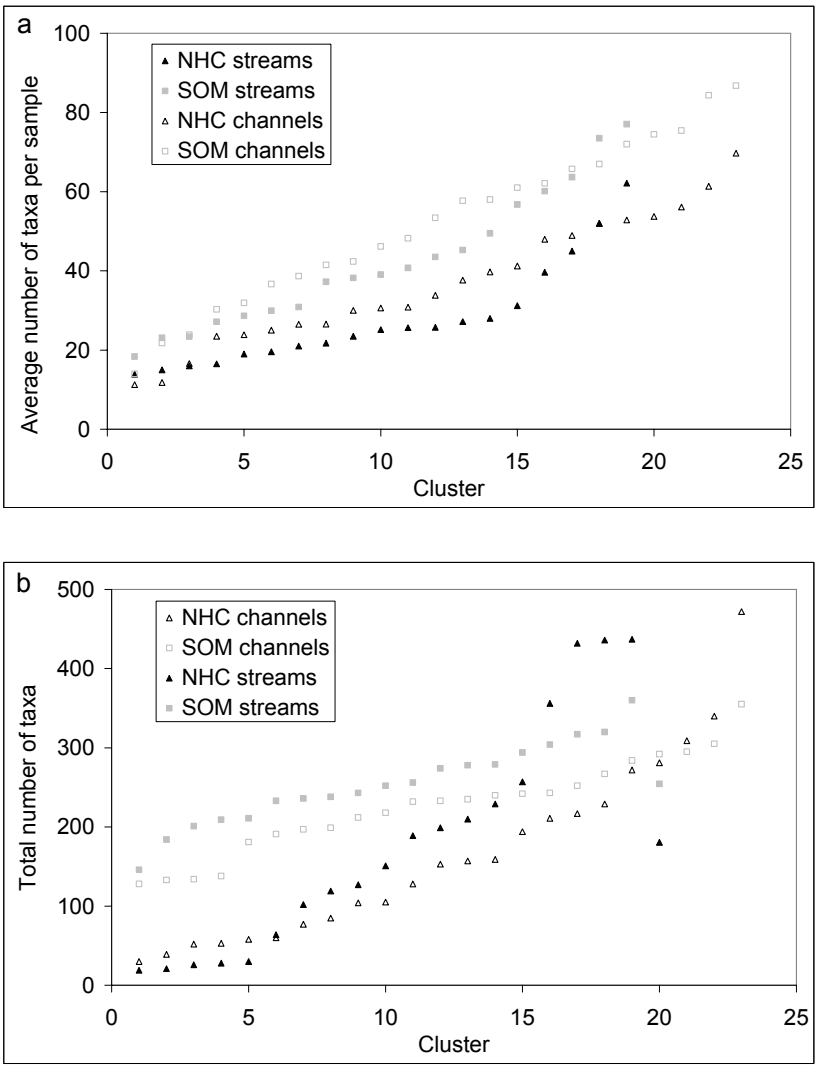

Fig. 5. The average number of taxa per site (a) and the total number of taxa per cluster (b) for stream and channel clusters (19/23 clusters) using SOM and NHC.

The choice of the classification technique and the options within the technique are important and strongly influence the results. Mangiameli *et al.* (1996) concluded that the SOM was superior to seven hierarchical clustering algorithms tested. They observed that the SOM classification is robust across all kind of data with different imperfections, such as outliers. In our study isolation values were higher for the NHC classification. However, it is not possible to state that one technique is better than the other because they focus on different characteristics of the data. The suitability of the classification depends directly on the application goal. The SOM technique results in a

more even distribution of the sites over the clusters. This results in higher resemblance and thus lower isolation values. But, on the other hand, the even distribution of the sites over the clusters can be a great advantage in the development of models for prediction of communities. The skew distribution of the NHC results is probably more close to the reality. In the data sets there are large groups of sites that are quite similar and originating from eutrophied channels or normalised streams, while more extreme or undisturbed situations occurred less frequently and therefore made smaller clusters. But, the separation of single sites is a problem in this technique, especially if these sites are not real outliers, but just the endpoints of large gradients.

It is hard to choose the right number of clusters within a technique. Probably there is not one best option. Both, using 40 and 19/23 clusters resulted in similar isolation values, meaning that the classifications were evenly distinct. Therefore, one should clearly keep the application of the classification in mind, and decide on the number of clusters using cluster tables in which the grouping of the sites is visualised.

Typifying taxa

The number of highly typifying taxa was higher in the NHC classification using 40 clusters, (235 for streams and 289 for channels) than using SOM (217 in streams and 214 in channels). About half of the highly typifying taxa were overlapping between NHC and SOM results (126 for streams and 148 for channels). Using 19/23 clusters the numbers of highly typifying taxa decreased for both techniques and both data sets. Probably, the fusion of the sites into fewer clusters did the number of taxa per cluster increase and less taxa became characteristic for a cluster. SOM classification had the same number of typifying taxa as the NHC classification for the channels and a higher number for the streams (131 for SOM and 91 for NHC). Thus, there was no general trend, neither for the number of typifying taxa resulting from a classification technique, nor for the number of typifying taxa in relation to water type (streams or channels). Using 19/23 clusters only 40 taxa were overlapping between both techniques for streams and 36 taxa for channels. The results were thus similar between streams and channels. The number of overlapping typifying taxa was small. This means that the classifications were based on different assemblages of taxa and sites, which confirms the results earlier in this paragraph. Typifying taxa that occurred in both classifications were taxa with high abundances and occurrence in many sites (Table 6). Taxa that were only typifying in the SOM classification also had relatively high frequency and abundance, while the ones that were highly typifying in the NHC classification were less abundant and less frequent. This means that NHC classifies also on less widely distributed taxa than SOM does.

Table 6. Number of sites in which indicator species occur and average abundance for three groups of indicator species, (1) only indicative in NHC, (2) only indicative in SOM, and (3) indicative in both techniques (stream data divided in 40 clusters).

	NHC		SOM		SOM and NHC	
	no. sites	average abundance	no. sites	average abundance	no. sites	average abundance
average	28	15	41	21	50	34
minimum	1	1	2	1	3	2
maximum	185	268	185	203	171	690
10 percentile	3	2	7	2	13	3
90 percentile	70	45	107	34	98	55

Classification with reduced taxa data

Distribution of the sites over the clusters

Table 7. Comparison of the distribution of the sites over the 40 and 19/23 clusters using different parts of the taxa data.

Data set	Deviation	Streams		Channels	
		40 output units	19 groups	40 output units	23 groups
SOM					
Indicator taxa	type a *	20	20	13	12
	type b **	20	21	13	12
Dominant taxa	type a	44	37	33	31
	type b	44	40	35	29
5 taxonomic groups	type a	54	45	52	52
	type b	55	49	57	56
NHC					
Indicator taxa	type a	16	15	15	17
	type b	15	20	19	25
Dominant taxa	type a	17	12	19	17
	type b	15	13	17	30
5 taxonomic groups	type a	25	24	31	25
	type b	27	22	34	38

* Type a deviation = % of sites that was in one group using all taxa, but put in different groups using the reduced taxa data.

** Type b deviation = % of sites that originates from different groups using all taxa and is included in the same group using reduced taxa data.

Using only part of the taxa data resulted in differences in site distribution over the 40 as well as the 19/23 output units for both stream and channel data. Using the SOM with indicator taxa the results were quite similar to those using all species, the deviation was 20% for streams and 12% or 13% for channels using 23 groups or 40 output units, respectively (Table 7). This was expected because the indicator taxa

were those taxa with a high maximum probability in the initial SOM including all taxa. For dominant species the errors were about 30%. Using 5 taxonomic groups, the distribution of the sites over the groups was very different from using the complete data set for streams as well as for channels, although the number of taxa in these data sets was higher than for dominant taxa. In most cases more than half of the sites were put in a different group. This means that using a part of the data including rare as well as common dominant species gives worse results than using a part of the data in which only dominant taxa occur. This is partly explained by the fact that most of the indicator taxa, which were of importance for the SOM classification were also dominant (148 and 207 taxa overlapped between indicator and dominant taxa for streams and channels respectively). The overlap between dominant or indicator taxa and the taxa of the 5 taxonomic groups was smaller. Thus, dominance plays a role in SOM classification, but not a large role, otherwise the errors for using only dominant taxa would have been smaller. Using larger groups by clustering the 40 output units into 19 and 23 groups for streams and channels respectively, the deviation if using reduced taxa data was similar.

Using NHC type a as well as type b deviations were both smaller in all cases than they were in the SOM results (Table 7). Only the NHC classification with the indicator taxa resulted in larger type a as well as type b deviations in comparison to the SOM results. This could be explained by the fact that the indicator taxa were chosen from the first SOM training and not from the NHC results. Using 5 taxonomic groups in the channel data resulted in the largest deviations of 34% and 38% for type a and type b deviations, respectively. This was similar to the SOM results. Because the errors were lower for dominant taxa, the NHC seems to cluster sites with similar abundances of dominant taxa.

Cluster characteristics

Although the distribution of the sites over the clusters differed strongly between complete and reduced data using the SOM, the isolation values of the resulting classification for channels did only slightly change (Fig. 6a). This means the classification results were not worse, they were only different. Also the reduction of the number of clusters from 40 to 19/23 did not result in different isolation values. Similar results were found for streams, isolation values varying between 0.61-0.68. Other researchers have reduced the number of taxa before analysis. Chon *et al.* (2000), for example, summed the species density into 7 selected taxa on high taxonomic level to avoid noise, caused by species with low densities. They assumed that this might have contributed to stabilising the process. However, we did not observe a change of the isolation values of the clusters, which indicates that reducing the number of taxa did not improve nor worsen the classification. Probably, this was caused by the fact that in our study taxa were deleted from the data. Chon *et al.* (2000) put many taxa

into one by adding their densities. Above that, they used taxa richness within the seven taxa and density of the taxa as input variables. This leads to a small but distinct data set with large gradients in densities and number of taxa.

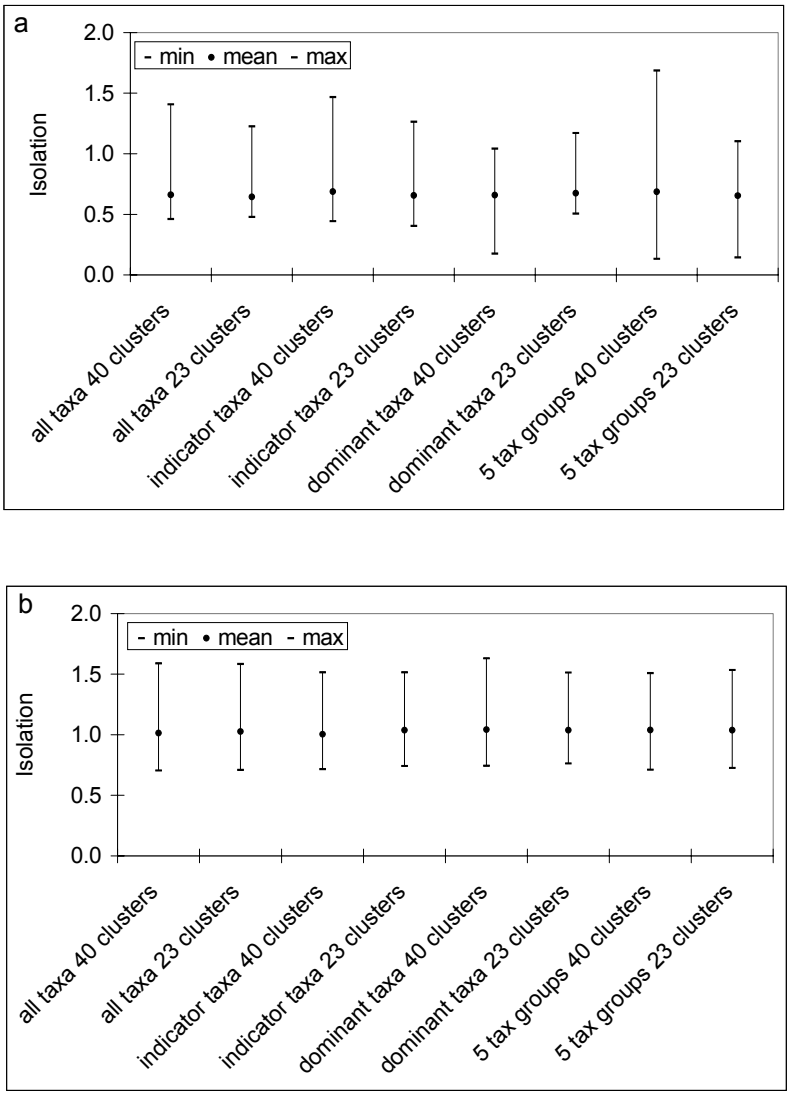

Fig. 6. Minimum, mean and maximum isolation values for the classification in 40 and 23 site groups using SOM (a) and NHC (b) for channel data.

Similar results were retrieved from the NHC classification of channel sites (Fig. 6b). For this technique, the results were less surprising, because the changes in distribution of sites over the clusters were much smaller than for the SOM results. The NHC classification had higher isolation values as was already observed in paragraph 3.2. Also the NHC classification keeps similar isolation values for the resulting clusters if another number of clusters was made or if reduced taxa data were used. Thus a different classification of the samples did not result in higher or lower isolation values. Similar results were observed for the stream data, isolation values ranged from 1.03 to 1.18.

In conclusion, for SOM only indicator taxa give similar results compared with using all taxa and for NHC indicator taxa as well as dominant taxa were useful. Indicator taxa are to be selected after a first classification analysis and are therefore not useful to reduce sampling and sorting costs. Dominant taxa could be useful if NHC is used. Excluding the rare taxa from data analyses could save costs but still there is a difference of 12 to 25% with the classification that included all taxa. Using only 5 taxonomic groups resulted in a completely different classification compared to using all taxa.

Still, the question remains whether the community is better or worse described by using all species data or only a selection remains. Schleiter *et al.* (1999) concluded that dimension-reducing pre-processing of the data in which the most indicative species are selected caused an increase of the generalisation performance of ANNs and a considerable reduction of the calculation effort. Many rare species are unlikely to be detected by sampling and, even when detected, the estimated abundances of such species are unreliable (Manté *et al.*, 1995). However, this was not confirmed by our study because the isolation values were not lower if all or a selection of taxa was used. Moreover, rare species appeared to be indicative for unimpacted sites and specific habitats (Nijboer and Schmidt-Kloiber, 2004).

Comparison between SOM and CCA

Comparing the SOM and CCA results for channels, Figs. 2, 7, and 8 show that the main gradients were similar between both techniques. The ordination diagram (Fig. 7) shows that chloride and conductivity explained the largest part of the variation in the data (these variables have the longest arrows). On the SOM the distribution of these variables is restricted to limited areas but the differences between these areas and the others are large (Fig. 2). Fig. 8 summarises the distribution of environmental variables on the SOM trained community data set in Figs. 2 and 4.

The other variables that are restricted to limited areas on the SOM are: intermittence, NH_4^+ and NO_3^-. In the ordination diagram these variables have shorter arrows than chloride and conductivity, thus they were of less importance. The arrow for intermittence is short, probably because it concerned only few sites and thus these

variables had less influence on the total analysis. However, in SOM this variable was of more importance, but only in a limited number of output units. The same goes for emergent vegetation, which was of no importance in CCA but characteristic in a small area on the SOM.

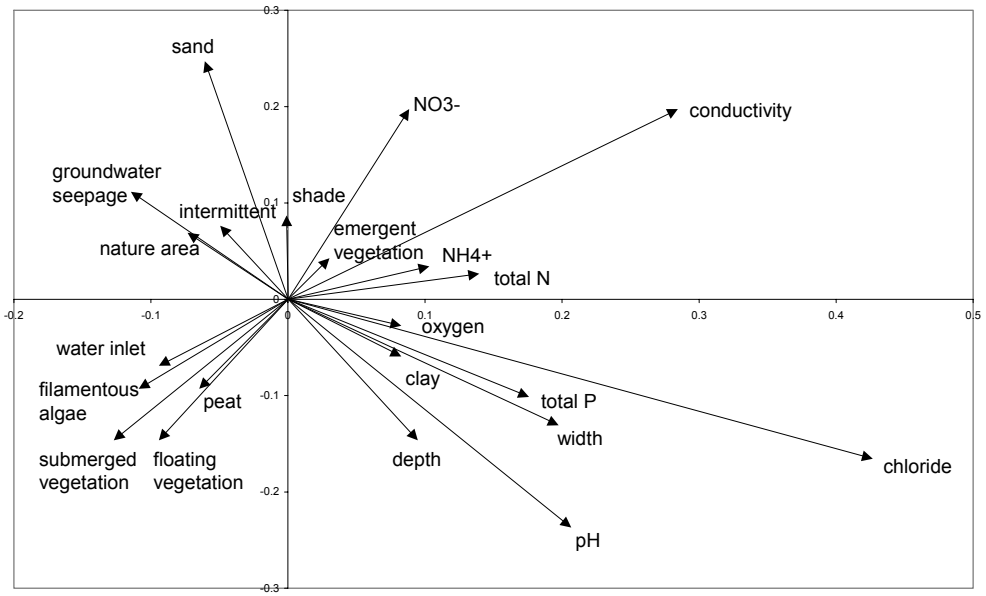

Fig. 7. Ordination diagram resulting from the Canonical Correspondence Analysis for channels.

Total phosphorus and width are also restricted to a limited area on the SOM map, but the number of sites in this area was higher, which explains the longer arrows in the ordination diagram. The gradient of these variables was longer. The variables that are widely spread on the SOM map have only moderately arrows in the ordination diagram. These variables had a smaller gradient, the differences between the sites were smaller. This is visible in the results of both techniques.

For streams, the patterns were even more similar (Figs. 4, 8, and 9). The variables NH_4^+, N-Kjeldahl, and total phosphorus are positioned together in the ordination diagram, represented by long arrows, correlated with the second axis, and also in one group, restricted to a limited area on the SOM map. The second group, consisting of silt, presence of dams, depth, and width is also recognisable as a group in the ordination diagram. These variables explained most of the variation on the first axis. They are distributed over a larger area on the SOM map than the first group but from the ordination diagram appears that they had long gradients in the data and were therefore important. The last group of variables that are widely spread over the SOM

show very small arrows on the ordination diagram. This means that these variables had values that did not differ much between sites. Their influence was only small. The middle group in Fig. 9 included variables that have long arrows in the ordination diagram, but pointing towards the left lower part of the diagram. These variables were important in about half of the SOM map. The ordination diagram shows that they represent large gradients.

Channels	Streams
chloride, conductivity, intermittent, NH_4^+, NO_3^-	NH_4^+, N-Kjeldahl, total phosphorus
width, total phosphorus, emergent vegetation	silt, dam, depth, width
sand, nature area, ground water seepage	NO_3^-, shade, meandering, natural profile, natural area, current velocity
floating vegetation, filamentous algae, submerged vegetation, peat	oxygen, sand
water inlet, depth, pH, total nitrogen, clay	chloride, conductivity, permanent, pH

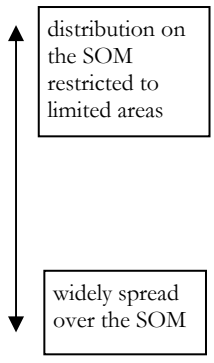

Fig. 8. Characteristics of the environmental variables according to their distribution on the SOM. Variables in one group have a similar distribution pattern on the SOM.

Although linking environmental variables to the SOM is an indirect technique and analysing the relationships between environment and species data in the CCA is a direct technique, both techniques showed similar results concerning the main gradients in the data. However, this should be tested with data sets including only smaller gradients.

The CCA focused more on the length and the direction of gradients while the SOM focused more on the distribution of variables over the clusters. Giraudel and Lek (2001) also concluded that it is not possible to control the direction of the gradients with the SOM. Many researchers used ordination in combination with classification to relate communities to environmental variables (e.g., Verdonschot, 1990). But the availability of both, classification and relating environmental variables to the clusters within one technique could be an advantage, especially, if a model to predict communities from environmental variables is going to be the next step.

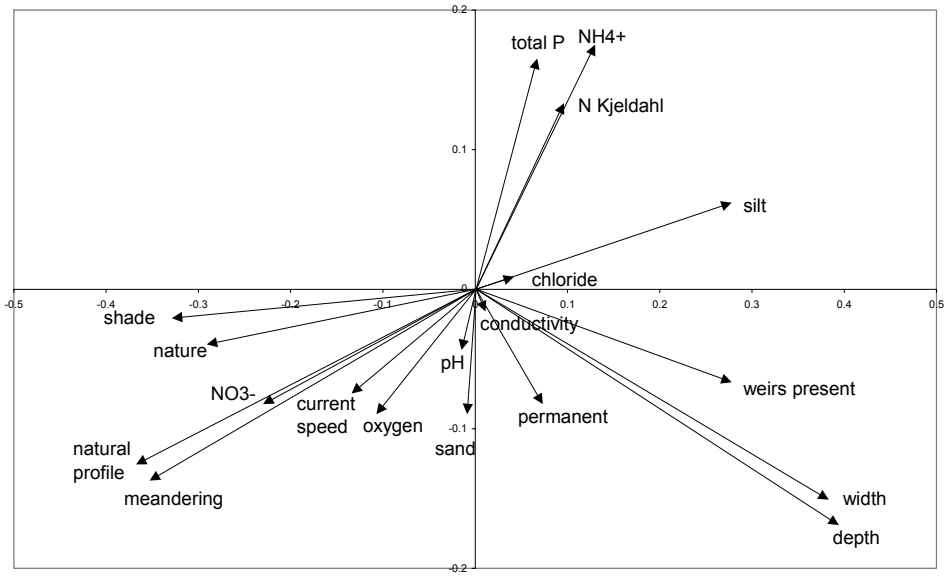

Fig. 9. Ordination diagram resulting from the Canonical Correspondence Analysis for streams.

Ordination with reduced taxa data

The sum of the eigenvalues of the first four axes decreased if only parts of the taxa data were used. This means that the gradient in the data became smaller. This trend was observed for streams as well as for channels (Table 8). This result was expected because the more taxa were excluded from the data the less variation was kept. The results confirm that with deleting taxa, information is deleted that is not represented by other taxa. For channels, the deviation in sum of eigenvalues was small for indicator taxa, larger for dominant taxa and largest for taxa from five taxonomic groups. In streams, dominant taxa showed the smallest deviation followed by indicator taxa and taxa from five taxonomic groups. This indicates that dominant species are more important in the stream data than they are in the channel data, although the stream data set with only dominant taxa is the stream data set with the lowest number of taxa.

The effect on the order of importance of the environmental variables as resulted from forward selection appeared to be small (Table 9). The results did vary between data sets but only for the variables of minor importance (all variables were significant, but the amount of variation in the data they explained differed). The most important variables were the same ones or were only exchanged with the next or previous variable in order of importance. Only, using dominant taxa in stream data resulted in an exchange of shade (the fourth variable) with nitrate (the eleventh variable). Probably, the taxa that were deleted were related to shaded waters. Although this

change in major variables, using dominant taxa did result in the smallest deviation of eigenvalues compared to the other partial data sets (Table 8).

Table 8. Eigenvalues of first, second, third and fourth ordination axes and sum of these four eigenvalues for complete and partial data sets as a result from Canonical Correspondence Analysis.

data set	eigenvalue 1st axis	eigenvalue 2nd axis	eigenvalue 3rd axis	eigenvalue 4th axis	sum of eigenvalues axes 1-4
streams all taxa	0.344	0.133	0.082	0.074	0.633
streams indicator taxa	0.339	0.130	0.076	0.064	0.609
streams dominant taxa	0.349	0.134	0.074	0.072	0.629
streams 5 tax groups	0.284	0.126	0.085	0.060	0.555
channels all taxa	0.257	0.146	0.085	0.068	0.556
channels indicator taxa	0.255	0.144	0.082	0.066	0.547
channels dominant taxa	0.250	0.134	0.071	0.061	0.516
channels 5 tax groups	0.260	0.090	0.070	0.062	0.482

Table 9. Results of forward selection in Canonical Correspondence Analysis. The columns with 'all taxa' show the order of importance of the environmental variables from high importance to low importance. The columns of the other data sets show the deviation from using all taxa in number of positions in order of importance (a positive number means the variable has become higher in order of importance, a negative number indicates a lower importance). The total deviance is the total number of exchanged positions.

	channels				streams		
all taxa	indicator taxa	dominant taxa	5 tax. groups	all taxa	indicator taxa	dominant taxa	5 tax. groups
chloride	0	0	0	depth	0	0	0
conductivity	0	0	0	natural profile	0	0	0
width	0	0	0	NH_4^+	0	0	0
sand	0	0	0	shade	0	-6	0
NH_4^+	0	0	0	width	0	0	0
pH	0	0	-1	oxygen	0	0	-6
natural area	0	0	+1	current speed	0	0	-4
floating vegetation	0	0	0	chloride	0	-1	0
water inlet	-1	0	-2	permanent	0	+1	-1
peat	+1	0	-8	pH	-1	-1	+1
depth	0	0	-2	NO_3^-	+1	+7	+4
total phosphate	0	-1	+3	conductivity	0	0	+6
NO_3^-	0	+1	+3	silt	0	0	0
clay	-1	-1	+2	natural area	0	0	-1
submerged vegetation	+1	+1	-1	meandering	0	-1	-1
shade	0	0	-3	sand	0	+1	+2
emergent vegetation	0	0	+2	total phosphate	0	0	-1
intermittent	-2	-4	+4	N-Kjeldahl	-1	0	+1
groundwater seepage	+1	+1	+2	dams	+1	0	0
filamentous algae	+1	+1	-1				
total nitrogen	0	+1	+1				
oxygen	0	+1	0				
total deviation	4	6	18	total deviation	2	9	14

The deviation of the order of environmental variables from using all taxa data was highest for using only 5 taxonomic groups, followed by using dominant species in both stream and channel data (Table 9). Using indicator species resulted in the most similar order of importance of the environmental variables. For streams this order is different from the order in extent of deviation of eigenvalues. This can be explained by the fact that using only dominant taxa another variable becomes more important while the extent of variation in the data remains similar.

Conclusions

Analysing community patterns appeared to be difficult and not objective. There are many techniques that could be used and the two examples in this study showed that different results are obtained with these techniques. A large percentage (50%) of the sites was clustered with other sites if non-hierarchical clustering was used instead of a self organising map or the other way around. Differences depend on the community characteristics on which the technique focuses. One technique is not always better than the other, one should choose the most appropriate technique depending on the goal of the study and the application of the classification. The SOM appeared to cluster sites with similar numbers of taxa and similar densities of the most dominant taxa. The NHC clustered on the similarity between all species, by which the abundance plays a major role. The number of taxa was of minor importance. Stream and channel data showed similar results, although the number of taxa per site was higher for channels, while the total number of taxa was lower.

The number of clusters that should be included in the classification can be chosen within the techniques. However, it is very hard to interpret which number of clusters is the most appropriate. It is useful to try classifications with different numbers of clusters and compare the isolation values but this will not automatically lead to the best solution. In this study the mean isolation value of the clusters appeared to be quite similar between the classifications of 40 and of 19/23 clusters. Therefore, it might be better to relate the number of clusters to the application goal of the classification.

Reducing the taxa data resulted in both techniques in another distribution of the sites over the clusters for both streams and channels. For the SOM the classification changed more than for the NHC, thus the results again depended on the technique that was used. The classifications had similar values for isolation, thus they were not worse than if the complete data were used. If reduction of the data is desirable one should at least compare the differences with the classification of the complete data to evaluate the suitability of the reduced taxa data. Therefore, it does not yet add to cost effectiveness in water management.

The relations with the environmental variables were quite comparable between SOM and CCA. Both techniques could be used together to get the most information out of the data. The main gradients were the same. The advantage of the SOM is that the environmental variables can be related to the clusters on the SOM. The CCA however, is better suitable for showing the length and direction of the gradients. Reducing taxa data reduced the amount of variation in the data but this reduction was only represented by less important environmental variables.

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7 The use of indicator taxa as representatives of communities in bioassessment

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Summary

1. Sampling and processing of benthic macroinvertebrate samples is time consuming and expensive. Although a number of cost-cutting options exist, a frequently asked question is how representative a subset of data is of the whole community, in particular in areas where habitat diversity is high (like Dutch surface water habitats).
2. Weighted averaging was used to reassign 650 samples to a typology of 40 community types, testing the representativeness of different subsets of data: (i) four different types of data (presence/absence, raw, ^{2}log- and ln-transformed abundance), (ii) three subsets of 'indicator' taxa (taxa with indicator weights 4-12, 7-12, and 10-12) and (iii) single taxonomic groups (n = 14) by determining the classification error.
3. ^{2}log- and ln-transformed abundances resulted in the lowest classification error, whilst the use of qualitative data resulted in a reduction of 10% of the samples assigned to their original community type compared to the use of ln-transformed abundance data.
4. Samples from community types with a high number of unique indicator taxa had the lowest classification error, and classification error increased as similarity among community types increased. Using a subset of indicator taxa resulted in a maximum increase of the classification error of 15% when only taxa with an indicator weight 10 to 12 were included (error = 49.1%).
5. Use of single taxonomic groups resulted in high classification error, the lowest classification error was found using Trichoptera (68%), and was related to the frequency of the taxonomic group among samples and the indicator weights of the taxa.
6. Our findings that the use of qualitative data, subsets of indicator taxa or single taxonomic groups resulted in high classification error implies low taxonomic redundancy, and supports the use of all taxa in characterising a macroinvertebrate community, in particular in areas where habitat diversity is high.

Keywords: classification, transformation, sample assignment, cost effectiveness, taxonomic group

Introduction

Growing concern for public health and the loss of aquatic integrity has resulted in the development of a number of systems to monitor and assess the ecological quality of inland surface waters (e.g. Armitage, Furse & Wright, 1992). For example, environmental managers need techniques to identify the present ecosystem status as well as to predict changes that might occur due to degradation or restoration measures. Using assessment systems for classifying the ecological integrity of freshwater waters has a long history (e.g. Metcalfe, 1989; Verdonschot, 2000). Benthic macroinvertebrates are commonly used in many assessment systems because they are recognised as one of the most reliable biological indicator groups in aquatic ecosystems (Hellawell, 1986).

Two types of macroinvertebrate assessment systems are commonly used; namely, multimetric and community approaches. In the multimetric approach a number of biotic or ecological indices/metrics are combined to form a multimetric index that is used to assess site quality. The first multimetric systems were developed by Karr *et al.* (1986) and the US Environmental Protection Agency (1988) using integrated ecological indices. In contrast to multimetric approaches, the community approach focuses on the complete macroinvertebrate community, using both taxa composition and environmental variables to assess or predict site quality (e.g. Wright, Furse & Armitage, 1993; Reynoldson *et al.*, 1995; Davies, 2000). Sites are classified into groups using clustering and ordination methods based on the similarity of taxonomic composition (Resh, Rosenberg & Reynoldson, 2000) and new samples can be classified by comparing them to the sites in the assessment system (e.g. Reynoldson *et al.*, 1995; Resh *et al.*, 2000).

In the Netherlands, the community-based assessment system 'Ecological characterisation of surface waters' (EKO) uses a typology of both reference and impacted sites of flowing and still waters (Verdonschot, 1990). The system can be used to assign a new sample to one of the 40 macroinvertebrate community types of different ecological quality (Verdonschot & Nijboer, 2000). EKO uses a combination of five assignment methods (Mahanalobis distance, Euclidean distance, weighted averaging, Czekanowski coefficient and squared Euclidean distance) to assign samples to their original community type (Verdonschot & Nijboer, 2000).

However, using an assessment system implies costs, since sampling, sorting and identifying macroinvertebrates are all time consuming processes. To reduce costs, it would be more efficient if the number of taxa that are necessary for assigning a sample to a community type and assess its quality could be reduced. Subsampling and the use of family- or genus-level identification are two methods commonly used to reduce costs associated with sample processing. They are, however, two of the most frequently criticised methods (King & Richardson, 2002). Subsampling is recommended by the US EPA's Rapid Bioassessment Protocols (Barbour *et al.*, 1999),

and has been important for reducing the costs associated with processing benthic macroinvertebrate samples (Barbour & Gerritsen, 1996). Subsampling may, however, result in taxa that are missed or not sorted in representative numbers, and for these reasons subsampling is not recommended for lowland streams and standing waters where habitat diversity is high and samples are heterogeneous, resulting in high numbers of low abundant taxa (Kimmins, 1973). The use of family- or genus- as opposed to species-level identification is also the subject of much debate (e.g. Lenat & Resh, 2001; Bailey, Norris & Reynoldson, 2001). Although a number of studies have shown little effect of varying taxonomic resolution on ecological classification (e.g. Bowman & Bailey, 1998), others have shown that species within genera have different responses to ecological conditions (e.g. Resh & Unzicker, 1975; Schmidt-Kloiber & Nijboer, 2004) and therefore recommend identification to the lowest practicable level. For example, it has been suggested that genus/species level data are needed when taxonomic or ecological diversity within families is large, or when areas have a diverse fauna (Bowman & Bailey, 1998; Hawkins *et al.*, 2000). Bailey, Norris & Reynoldson (2001) concluded that precise identification is needed if: (1) differences between sites are small, (2) rare species are important and (3) more than three ecological quality classes are to be used in ecological classification.

As both higher taxonomic resolution and subsampling are not recommended in heterogeneous habitats with many rare taxa (like in the Netherlands) other cost-reducing approaches are needed. Marchant (1999) and Gauch (1982) postulated that some ecological data is redundant and hence can be excluded from analysis without loss of information. For example, according to Pearson, Gray & Johannessen (1983) both rare and opportunistic taxa can be considered redundant, and these authors concluded that intermediate taxa may be the best indicators. Accordingly, taxa indicative of a specific community type are those that occur in high frequencies and high abundances in comparison to the other community types. Use of only such indicator taxa might be a way to reduce costs without loss of important ecological information. Another cost-reducing endeavour might be the use of a single taxonomic group. For example, in bioassessment, metrics such as EPT (Lenat, 1988), % Diptera or the proportion of *Limnodrilus hoffmeisteri* Claparede, 1862 versus all other Tubificidae (Brinkhurst 1966) are used to assess water quality, implying the usefulness of a single taxonomic group as an indicator of ecological change. A third cost-cutting option is the use of qualitative instead of quantitative data. Gauch (1982) concluded that qualitative differences in taxa compositions between sites are often large, and indeed a commonly used metric in assessment and biodiversity studies is simply the number of taxa (e.g. Weber, 1973; Rosenberg & Resh, 1993). Other examples of the use of binary data are the Biological Monitoring Working Party (BMWP) score system (Armitage *et al.*, 1983), the rapid assessment protocols (Plafkin *et al.*, 1989) and some community similarity indices such as the Jaccard Index (Jaccard, 1912). Resh & Jackson (1993)

concluded that whether quantitative or qualitative approaches are to be used depends on the purpose of the study and the sensitivity required.

The key question of this study was to determine if a subset of taxa can be used to represent the macroinvertebrate community present. To test this assumption we tested if samples were assigned to their original community type using: (1) qualitative data, (2) different subsets of indicator taxa and (3) single taxonomic groups.

Methods

Weighted averaging

A macroinvertebrate community typology (EKO) was developed by Verdonschot (1990) using samples of macroinvertebrate communities from still and flowing waters (650 sites) in the Netherlands (Verdonschot, 1990; Verdonschot & Nijboer, 2000). Macroinvertebrates were sampled using a standard macroinvertebrate sampling protocol (Verdonschot, 1990). In brief, a sample of 1.5 m^2 was taken with a pond dip net where the sample effort was divided proportionately over the habitats present in the surface water. All individuals were sorted from the sample and identified to species level if possible. The data set consisted of 854 taxa, including 75% species, 21% species-groups or genera and 4% taxa at higher taxonomic levels. Physical variables were measured at the sites and a water sample was taken for chemical analyses. The typology was developed using Preston's transformation (^{2}log(x+1)) (Preston, 1962; Verdonschot, 1990) of the macroinvertebrate abundances at the sites in combination with environmental variables (physical and chemical) using clustering (Van Tongeren, 1986) and ordination (CANOCO, Ter Braak, 1988). The macroinvertebrate community typology was included in a computer program (named: 'EKO'), which was designed to assign a new sample of a macroinvertebrate community from a surface water to one of the 40 community types (Verdonschot & Nijboer, 2000).

One of the assignment techniques within the EKO program is weighted averaging (Sládeček 1973). Weighted averaging uses the indicator weights for each taxon within a community type:

$$T_K = \frac{\sum_{i=1}^{i=p} (t_{iK} * n_i)}{\sum_{i=1}^{i=p} n_i}$$

where T_K is the typifying index of a sample for community type *K*, t_{iK} is the indicator weight of taxon *i* for community type *K*, n_i is the total number of individuals of taxon *i* in the sample and *p* is the total number of taxa in the sample.

The indicator weight of a taxon for a community type (t_{iK}) was calculated using constancy, fidelity and concentration of abundance (Boesch, 1977; Verdonschot, 1984). Constancy is defined as the number of occurrences of a taxon in a community type divided by the number of sites in the community type. Fidelity is the degree to which a taxon prefers a community type, defined as the ratio of the relative frequency of a taxon in a community type and its overall relative frequency. Concentration of abundance is the average abundance of a taxon in a community type divided by its average overall abundance. Constancy, fidelity and concentration of abundance were combined to assign an indicator weight to a taxon per community type according to the values given in Table 1. The indicator weight related to the combination of the three characteristics was extracted from this table by checking, in order of occurrence, whether each characteristic was in accordance with the limits indicated. For example, if constancy is 0.29, fidelity 5.6 and concentration of abundance 6.2, the indicator weight is 10 (third row in Table 1). The indicator weights vary from one to 12 (Verdonschot, 1990), and for each of the 40 community types a taxa list with the indicator weight for each taxon was constructed. The taxa in a community type were divided into four indicator categories (Table 1).

Table 1. Indicator weights and categories used in EKO classification of Dutch waterbodies. An indicator weight is assigned to a taxon when, in accordance to occurrence from top to bottom in the table, constancy, fidelity and concentration of abundance are all higher than the boundary indicated.

Constancy	Fidelity	Concentration of abundance	Indicator weight	Indicator category
> 0.50	> 3	> 5	12	high
> 0.40	> 4	> 4	11	high
> 0.25	> 5	> 5	10	high
> 0.50	> 2	> 4	9	moderate
> 0.40	> 3	> 3	8	moderate
> 0.25	> 4	> 4	7	moderate
> 0.50	> 1	> 3	6	low
> 0.40	> 2	> 2	5	low
> 0.25	> 3	> 3	4	low
> 0.50	> 1	> 1	3	indifferent
> 0.25	> 1	> 1	2	indifferent
> 0.00	-	-	1	indifferent

Using weighted averaging, we reassigned the original 650 samples from the EKO database to the 40 community types. To evaluate the suitability of different methods

tested here we calculated the percentage of the samples that were assigned to their original community type (% 'correct' classification). Different transformations of taxa abundances, calculation methods for weighted averaging and subsets with groups of indicator taxa and single taxonomic groups were evaluated.

Data transformation

Four types of data transformation were tested to determine which transformation of taxa abundances resulted in the highest percentage of samples assigned to their original community type: (1) numbers of individuals (abundance), (2) presence/ absence, (3) logarithmic transformation (ln(x+1)) and (4) Preston's transformation (^{2}log(x+1)) (Preston, 1962; Verdonschot, 1990). All four transformations were tested using the complete taxa data set and the transformation that resulted in the highest percentage of samples assigned to their original community type was used in the subsequent analyses.

Calculation methods

A community type is based on a number of macroinvertebrate samples, each of which can have a slightly different taxa composition. All taxa of a sample occur in the taxa list of the original community type (having an indicator weight > 0), but not all taxa occur in each sample. This among-sample variation in taxa composition makes assignment of a sample to another community type possible. Calculation of the 'typifying index' was done for all 40 community types. For the original community type all taxa from the sample are present, whilst for the other 39 community types the sample can include taxa that do not occur in the respective community type. We tested if inclusion of these taxa in the 'p' of the weighted averaging formula resulted in lower classification error.

Weighted averaging was calculated by: (1) dividing the numerator by the sum of the abundances of all taxa in the sample, i.e. including those taxa which have indicator weight zero for the community type (the taxa that are in the sample but not in the taxa list of the community type) (method 1) and (2) dividing the numerator by the sum of the abundances of only the taxa in the sample that are also listed for the community type (i.e. taxa which have an indicator weight >0 for the community type) (method 2). Both approaches were tested using the complete taxa data set and subsets with different indicator groups. The method that resulted in the highest percentage of samples assigned to their original community type (the lowest classification error) was used for testing the effect of single taxonomic groups on classification error.

Subsets of indicator groups

Reassignment of the samples to the typology was done using four subsets of taxa for each sample: (1) all taxa present in the sample, (2) only taxa from the sample with a

low to high indicator weight (4-12) for the respective community type, (3) only taxa from the sample with a moderate to high indicator weight (7-12) for the respective community type and (4) only taxa from the sample with a high indicator weight (10-12) for the respective community type. Differences between community types were calculated as the percentage of the samples from a community type that were re-assigned to the community type. Additionally, these percentages were also related to the number of taxa in each of the four indicator groups (indifferent taxa, taxa with low, moderate and high indicator weights) in the taxa list of the community type using correlation (Legendre & Legendre, 1998):

$$correlation(x,y) = \frac{\sum (x-\bar{x})(y-\bar{y})}{\sqrt{\sum (x-\bar{x})^2 \sum (y-\bar{y})^2}}$$

where x is the number of indicator taxa in the group analysed and y the percentage of samples of a community type re-assigned to that community type.

Subsets of a taxonomic group

We tested if use of taxa from a single taxonomic group resulted in a similar percentage of samples assigned to their original community type compared to the use of all taxa. The taxonomic groups were defined at a high taxonomic level to avoid a too strong reduction of the number of taxa in the subset of a sample: Ephemeroptera, Plecoptera, Trichoptera, Chironomidae, Acarina, Bivalvia, Coleoptera, Diptera (Chironomidae excluded), Gastropoda, Heteroptera, Hirudinea, Crustacea, Odonata and Oligochaeta. Each taxonomic group was tested using all taxa belonging to the respective taxonomic group. Stream (S) and pond (P) community types were used because they had a high percentage of samples assigned to their original community type using all taxa (74% and 72%, respectively).

Results

Analysis of different data transformations on classification error showed that Preston's transformation resulted in the highest (66.1%) and presence/absence data in the lowest (56.6%) percentage of samples assigned to their original community type. Raw and ln-transformed abundance data resulted in 63% and 65.9%, respectively, of correct classification.

The percentage of samples assigned to their original community type was highest (66.1%) when all taxa (with indicator weight 0 to 12) were included and when calculation method 1 (dividing the result of the numerator by the sum of the

abundances of all taxa in the sample) was used (Table 2). Use of fewer taxa (restricted to taxa with a higher indicator weight) resulted in fewer samples that were assigned to their original community type. For example, including only taxa with an indicator weight 10-12 resulted in 50.9% of the samples being assigned to their original community type. When the numerator of the weighted averaging formula was divided by only the taxa that occurred in both the sample and in the taxa list of the respective community type, the percentage of samples assigned to their original community type was even lower. Correct classification ranged from 40.3% if all indicator groups (indicator weights 1-12) were included to 0.5% if only taxa with an indicator weight higher than six were included.

Table 2. Percentages of original EKO samples that were assigned to their original community type using different subsets of indicator species. Abundances were transformed using Preston's ^{2}log transformation. In calculation method 1 the numerator of the weighted averaging formula is divided by the sum of the abundances of all taxa in the sample and in method 2 the numerator of the weighted averaging formula is divided by the sum of the abundances of the taxa in the sample that are also listed for the community type.

	Calculation method	
Subset	1	2
species with indicator weights 1-12	66.1	40.3
species with indicator weights 4-12	61.5	1.1
species with indicator weights 7-12	58.7	0.5
species with indicator weights 10-12	50.9	0.6

The percentage of samples that were assigned to their original community type varied strongly between the community types; a minimum of 13% for community type R9 (lower reaches of regulated streams) and a maximum of 100% for 11 community types (Fig. 1). The average for all community types was 69% (standard deviation 29%). The percentage of samples assigned to their original community type was positively related to the number of indicator taxa in the taxa list of the community type (Table 3). Regression showed that the number of taxa with moderate and high indicator weights together resulted in the highest correlation coefficient (0.69). The total number of taxa with low, moderate and high indicator weights also resulted in a high correlation coefficient (0.64). However, there were exceptions. The community types P1 and P2 had similar numbers of indicator taxa as P5 and P6, but the percentage of samples assigned to their original community type was higher for P1 and P2 than for P5 and P6. The number of taxa with a high, moderate or low indicator weight were all weakly correlated with the percentage of samples assigned to their original community type (correlation coefficients of 0.21-0.41). A high number of indifferent taxa and a high total number of taxa in a community type showed no correlation with the percentage of samples assigned to their original community type.

Table 3. Correlation between the number of taxa in a community type and the percentage of samples assigned to their original community type.

Indicator categories	Correlation coefficient
indifferent taxa (1-3)	-0.05
taxa with low indicator weights (4-6)	0.34
taxa with moderate indicator weights (7-9)	0.21
taxa with high indicator weights (10-12)	0.41
taxa with moderate or high indicator weights (7-12)	0.69
taxa with low, moderate, or high indicator weights (4-12)	0.64
all taxa (1-12)	0.05

The overall percentages of samples assigned to their original community type using a single taxonomic group were low (e.g. a maximum of 32% for Trichoptera) (Table 4). For a number of community types none of the samples were assigned to their original community type using single taxonomic groups (e.g. Chironomidae for samples from community types S2, S3, S7, S13, P5 and P9). Conversely, for some community types all samples were assigned to their original community type using single taxonomic groups (e.g. 100% using Coleoptera for community type S13). For some community types, samples were not assigned to their original community type using any of the single taxonomic groups (e.g. from S5 or P9), and some taxonomic groups resulted in a higher percentage of samples assigned to their original community types than others. For example, Trichoptera and Chironomidae resulted in the highest (32% and 30%, respectively) and Bivalvia and Plecoptera in the lowest (1% and 2%, respectively) overall percentages of samples assigned to their original community types. This finding was mainly related to the total number of taxa within these taxonomic groups (Table 5), indicating that the mean number of individuals per sample was less important for correct classification (e.g. Trichoptera). However, not all groups with a high number of taxa and many individuals resulted in a high percentage of samples assigned to their original community type (e.g. Gastropoda). This might be related to the number of indicator taxa within this group, which was low for the pond and stream community types (Table 6).

Some taxonomic groups resulted in high percentages of correct classification for some community types (e.g. Heteroptera in S13 (91%) and P9 (92%)). However, this finding was not related to the number of indicator taxa within the taxonomic group for these types, as they were low (two and three for S13 and P9, respectively). This was also shown by the Coleoptera, which had a high number of indicator taxa in S13, P1, P3, P4, and P5, while the percentages of samples assigned to their original community type differed from 45% for P5 to 100% for P1 and S13.

Table 4. Percentage of samples assigned to their original community type for each taxonomic group and each pond (P) and stream (S) community type (using calculation method 1, Preston's ^{2}log transformation and including all taxa in the respective taxonomic group).

	Taxonomic group													
Community type	Acarina	Bivalvia	Chironomidae	Coleoptera	Crustacea	Diptera (excl. Chironomidae)	Ephemeroptera	Gastropoda	Heteroptera	Hirudinea	Odonata	Oligochaeta	Plecoptera	Trichoptera
S1	50	0	78	53	50	72	0	0	0	0	0	0	38	61
S2	20	0	0	0	0	40	0	0	0	0	0	20	0	60
S3	0	0	0	0	0	60	0	0	0	0	0	40	0	0
S4	0	0	24	0	0	14	0	0	11	0	0	71	0	0
S5	0	0	18	0	0	0	0	0	0	0	0	55	0	0
S6	58	0	100	0	0	45	0	0	0	0	0	33	0	40
S7	33	0	0	16	11	0	41	0	7	0	7	11	0	58
S9	0	0	25	0	0	17	0	0	0	0	0	0	0	0
S10	60	15	36	0	0	0	0	60	0	58	0	32	0	0
S12	0	0	43	14	0	0	0	0	0	0	0	43	0	100
S13	0	0	0	100	0	0	0	0	91	0	0	0	0	0
P1	11	0	78	100	0	89	0	0	0	0	0	0	0	0
P2	53	0	7	27	0	33	86	0	67	0	67	50	0	79
P3	13	0	100	55	0	50	0	0	64	0	36	0	0	0
P4	45	0	54	76	0	86	9	70	50	62	72	50	0	45
P5	0	0	0	45	0	22	0	0	0	0	17	0	0	0
P6	0	0	4	7	0	0	0	0	0	0	61	4	0	70
P7	43	0	43	0	0	33	86	0	14	0	14	0	0	57
P8	0	0	11	0	0	0	29	0	18	79	9	7	0	59
P9	0	0	0	0	0	0	0	0	92	4	0	0	0	0
P11	67	0	50	50	0	0	0	0	42	0	0	0	0	9
total % samples	23	1	30	24	4	23	12	10	24	17	17	20	2	32

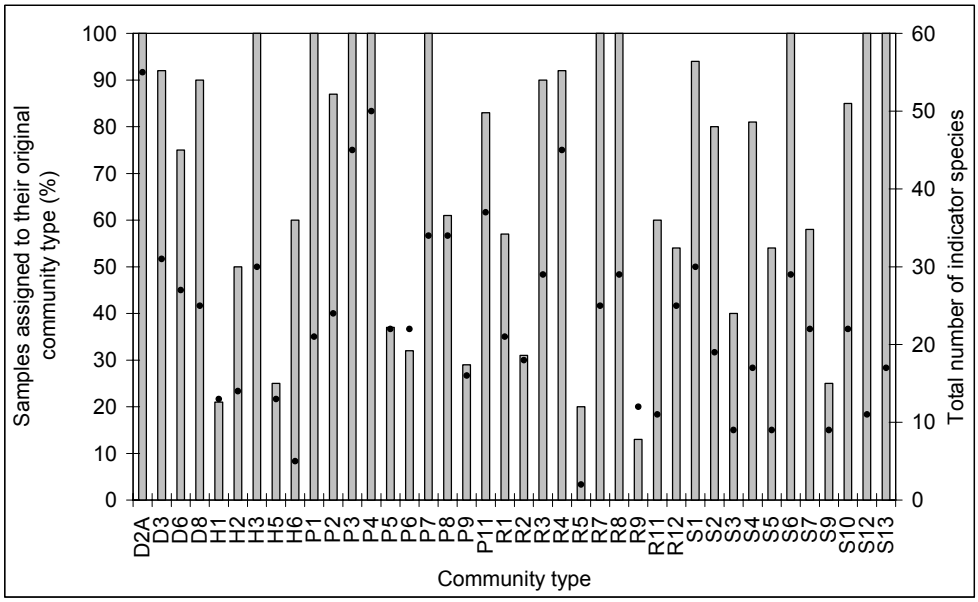

Fig. 1. Percentage of samples from a community type reassigned to the same community type using weighted averages including all taxa in the samples, Preston's ^{2}log transformation and method 1 (bars) and the number of taxa with an indicator weight > 3 for the community type (dots).

Table 5. Total number of taxa, mean number of taxa and individuals per sample and number of samples for each taxonomic group for pond (P) and stream (S) community types.

	total number of taxa		mean number of taxa per sample		mean number individuals/sample		number of samples	
	P	S	P	S	P	S	P (167)	S (142)
Acarina	112	45	6	3	48	20	141	75
Bivalvia	6	3	1	1	53	88	60	67
Chironomidae	78	78	13	9	313	189	167	140
Coleoptera	122	104	8	5	40	28	162	138
Crustacea	8	5	2	2	78	264	111	111
Diptera	36	41	3	4	60	35	156	131
Ephemeroptera	9	12	2	2	311	62	143	58
Gastropoda	31	25	7	3	239	87	129	104
Heteroptera	54	21	5	2	60	8	157	73
Hirudinea	10	11	4	2	46	15	133	90
Odonata	27	10	3	2	51	5	145	32
Oligochaeta	43	32	5	6	109	447	154	140
Plecoptera	1	3	1	1	10	109	2	88
Trichoptera	45	45	4	3	32	36	134	103

Table 6. Numbers of taxa with an indicator weight > 3 per taxonomic group and community type.

	Taxonomic group													
Community type	Acarina	Bivalvia	Chironomidae	Coleoptera	Crustacea	Diptera (excl. Chironomidae)	Ephemeroptera	Gastropoda	Heteroptera	Hirudinea	Odonata	Oligochaeta	Plecoptera	Trichoptera
S1	2		10	1	2	7						1	2	5
S2	3		4		1	2	1			1		4		3
S3			2	1		3						2	1	
S4			5			5						5	1	1
S5			5									4		
S6	6		14			3						4		2
S7	4	1	3	2	1	1	1		1	1		1		4
S9			4	1		3			1					
S10	2	1	5	4				3		2		3		
S12			2	3		1						2		3
S13				13		1		1	2					
P1	1		4	11		4						1		
P2	1		4	4		3	1		3		4			2
P3	1		10	10		6	1		12		2	1		2
P4	4		3	17		6	1	3	8	2	2	2		1
P5	1		1	13		2		5						
P6	1		5	2		2		1		1	2	1		6
P7	7		11	1		1	4		5		1			4
P8	1		7	2		2	1	2	5	4	2	2		4
P9			5		1			1	3	3		2		1
P11	11		9	3	1			1	6	1	1			3

Discussion

It has been postulated that qualitative differences in species composition among sites are more important than quantitative differences for classification (Gauch, 1982). The findings of our study did not support this conjecture, but showed that use of qualitative data resulted in a decrease of the percentage of samples assigned to their original community types by 10%. The use of raw abundances, as opposed to ln- or Preston-transformed abundances, resulted in a decrease of only 3%, probably because the ratio between the densities of taxa was retained. Whether the use of qualitative or quantitative data results in lower classification error is equivocal. For example, Reynoldson *et al.* (1997) suggested that use of presence/absence data in AUSRIVAS resulted in low classification error, while others have suggested that inclusion of density (as used in BEAST) may improve sensitivity for detecting impairment (Resh, Rosenberg & Reynoldson, 2000). Our finding that use of presence/absence data

resulted in higher classification error may, however, be due to our use of both reference and degraded sites in the typology. Abundance data are often necessary to detect impact, because some widespread species, which also occur at reference sites, can become dominant at polluted sites (Hellawell, 1978). For example, if such species have an indicator value for a degraded community type, the typifying index will be more influenced if abundance data are included, resulting in greater among-community type differences.

Dividing the numerator of the weighted averaging formula by all taxa abundances in the sample (method 1) gave the highest percentage of samples assigned to their original community type, indicating that taxa with no or low indicator weight for a certain community type may be important to 'increase' the dissimilarity of a sample, in particular if indicator taxa overlap between community types. Communities are not only characterised by the presence of some taxa, but also by the absence of other taxa (Kothe, 1962). If many taxa in a sample have no or low indicator weight for the respective community type, the typifying index for that community type will be low. However, these same taxa might have a high indicator weight for other community types (resulting in a high typifying index). Hence, including these taxa in both index computations should result in greater distinction between community types.

Excluding redundant information from the samples by excluding common (i.e. taxa that occur in most community types) and rare taxa (defined here as taxa that occur scattered in few samples randomly over the community types, sensu Gauch, 1982), should emphasise the indicator taxa and thus the differences between community types. Moreover, using only indicator taxa for identifying the community of a sample should reduce the problem of noise, because scattered rare species are excluded (Gauch, 1982). Consequently, we expected that excluding redundant information would result in clearer separation between community types and improve classification and reduce the macroinvertebrate data needed for assigning a new sample to a typology. Our findings did not support this conjecture however. Indeed, the percentage of samples assigned to their original community type was highest (66%) when all taxa present in a sample (with indicator weights ranging from 0-12) were used. Thus, including only indicator taxa did not result in a higher percentage of samples assigned to their original community type. This result was also not expected since the weighted averaging technique was developed for using indicator taxa.

There are several possible explanations for why the use of only indicator taxa did not result in lower classification error. Firstly, the indicator taxa used here might not be distinctive enough to clearly separate the community types. Secondly, both the occurrence and abundance of the taxa in a community type in relation to other community types were used to assign indicator weights to the taxa. Abundance was included because differences between some of the types were mainly expressed by differences in abundances. Communities are not discrete, but constitute a continuum

along an environmental gradient (Curtis, 1959) and as the species composition of communities shifts slowly along an environmental gradient abundances of taxa increase or decrease before species are replaced by others (Verdonschot, 1990). Hence, species overlap between community types, especially ecologically similar types, and some taxa may have high indicator weights for more than one community type. For example, four of the taxa with high indicator weights for community type H1 also had high weights for H3, and five taxa with high indicator weights for H1 also had high weights for S1 (Verdonschot, 1990). This results in only one unique indicator species with an indicator weight of ten for H1, which may have resulted in the low percentage of samples from H1 assigned to this community type. This overlap in community composition was not surprising because the community from H1 (helocrene springs), overlaps with the community of S1 (spring brooks). H3 communities include slightly acidic springs (hence the small difference between H3 and H1 community types), and taxa indicating acid conditions are characteristic of this type. In addition, both S1 and H3 have a number of additional indicator taxa compared to H1. Therefore, a higher percentage of samples of these community types were assigned to these community types and overlap existed mainly with one or two of the most similar community types. Thus, samples with indicator taxa that occur in two community types, where some of the taxa belong to one and some to another community type, are difficult to assign unambiguously.

The number of taxa with moderate or high indicator weights was correlated to the percentage of samples correctly classified (to their original community type), implying that the method used here for assigning the indicator weights was robust. The number of taxa with low indicator weights did not improve the correlation between the number of indicator taxa and the percentage of samples assigned to their original community type. These community types were often 'unfavourable' environments, such as acid waters (e.g. P3 and P4) or community types that were relatively unimpacted (e.g. P7, S6, or D2A). According to Southwood (1977), species in unfavourable environments need specific adaptations and are therefore indicative, whereas species in favourable environments lack specific adaptations. In more favourable environments biotic interactions such as competition and predation are considered to play a major role, resulting in high natural variation, more overlap of (indicator) species and less distinct community types. Our results showed that as the differences between community types decreased it became more difficult to unambiguously assign a sample to the typology. Consequently, if the number of indicator taxa is low, it is important to also include taxa with no or low indicator weights to increase the distinction between community types.

Stricter criteria for assigning indicator weights (e.g. a higher ratio between abundance/occurrence in the community type in relation to other types) should result in less overlap of indicator taxa between community types, but also lower the total number of potential indicator taxa. It remains to be tested whether stricter criteria will

result in a higher percentage of samples assigned to their original community type. That some taxa used in our study were not identified to species might have resulted in 'artificial' overlap between community types, resulting in less accurate indicator weights and higher classification error. Another reason for the high classification error using only indicator taxa might be that redundancy in macroinvertebrate data was not as high as was expected and as has been suggested by others (Marchant, 1990, 1999; Gauch, 1982). For example, Cao, Williams & Williams (1998) showed that rare species are important, and Nijboer & Schmidt Kloiber (2004) recently found that excluding taxa with low abundances and/or with small distribution ranges resulted in higher classification error. Moreover, Nijboer *et al.* (2005) showed that developing a typology using indicator taxa only resulted in a different classification than if all taxa were used. Taxa with low abundance or occurrence may have a large influence on classification error. Similarly, common taxa (over the whole data set) that vary in abundance in different community types may be important in classifying community types that have a low number of indicator taxa. The finding that presence/absence data resulted in a decrease in the percentage of samples assigned to their original community type indicates that differences in abundances were important. In particular, the large differences in abundances of common species among the community types and abundances of overlapping indicator species can be important for classification error, in particular if differences in the species composition between community types are more subtle and the scale of the typology is finer (Marchant, 1999; Giraudel & Lek, 2001).

Our study showed that the use of a single taxonomic group resulted in a lower percentage of samples classified to their original community type than using all taxa in a sample. Chironomidae and Trichoptera gave the best results (e.g. maximum of 32% for Trichoptera), and both of these groups had the highest number of taxa, and Chironomidae also had high abundances, in the samples. This indicates that not only the number of taxa, but also the number of indicator taxa within the taxonomic group in the total dataset are important for classification error. These findings also lend support to an earlier study by Hawkins & Norris (2000) who showed that models based on Chironomidae taxa alone (although comprising 31% of the total number of taxa) performed poorly in discriminating between test and reference sites. Thus, even using a single taxonomic group with a high number of taxa, may result in a low percentage of samples assigned to the original community type. However, other studies have found the use of single taxonomic groups to be robust in discriminating impact. For example, Bauernfeind & Moog (2000) successfully assessed rivers and streams using mayfly taxa. The use of mayflies in our study mayflies gave poor results, which is probably because they are poorly represented in Dutch water bodies. Combinations of taxonomic groups may, on the other hand, be useful for classifying impairment. For example, Graça, Coimbra & Santos (1995) showed that differences in classification were small when aquatic insects were used instead of the whole

macroinvertebrate community. Moreover, Hewlett (2000) showed that a combination of Ephemeroptera, Plecoptera and Trichoptera (EPT) taxa yielded similar results for classification of water bodies. The number of EPT taxa is successfully used in rapid bioassessment systems (Lenat, 1988). In the Netherlands, Ephemeroptera and Plecoptera are poorly represented and our results showed that using either of these groups resulted in a low percentage of samples assigned to their original community type. Consequently, our findings imply that the relative success of using a single taxonomic group or assemblage may depend on the region and the water type.

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Appendix 1 Characterisation of the macroinvertebrate community types included in the EKO assessment program

The types are named after their abiotic conditions (Verdonschot and Nijboer 2000)

Helocrene springs

H1 oligo- to β-mesosaprobic helocrene springs
H2 temporary or desiccating, neutral to slightly acid, β-mesosaprobic seepage marshes
H3 neutral to slightly acid, oligo- to β -mesosaprobic helocrene springs
H5 slightly acid, oligo- to β-mesosaprobic, oligo-ionic helocrene springs
H6 temporary, acid, oligo-ionic, oligo- to β-mesosaprobic seepage marshes

Streams

S1 oligo- to β-mesosaprobic spring streams
S2 permanent, rainwater-fed, β-mesosaprobic upper reaches of natural streams
S3 temporary, α-mesosaprobic, small upper reaches of natural streams
S4 temporary, β-mesosaprobic upper reaches of natural streams
S5 polysaprobic upper and middle reaches of natural and regulated streams
S6 α-mesosaprobic middle reaches of semi-natural streams
S7 α-mesosaprobic middle reaches of regulated streams
S9 the summer aspect with α-meso- to polysaprobic conditions of temporary upper reaches of natural streams or temporary, α-meso- to polysaprobic regulated streams
S10 temporary, α-mesosaprobic, flowing upper reaches of regulated streams or ditches
S12 temporary, slightly acid, α-mesosaprobic upper reaches of regulated streams or ditches
S13 the summer aspect with α-mesosaprobic conditions of temporary, small upper reaches of natural streams

Ditches

D2A permanent, β-meso- to α-mesosaprobic, small, shallow ditches
D3 permanent, α-mesosaprobic, shallow, small ditches or stagnant regulated streams
D6 acid, oligo-ionic, α-mesosaprobic to polysaprobic small ditches
D8 temporary, very slightly flowing, α-meso-ionic, α-mesosaprobic small ditches

Rivers and canals

R1 β-meso- to α-mesosaprobic, medium-sized to large very slowly flowing lower courses of streams and rivers
R2 β-meso- to α-mesosaprobic, large ditches and small canals on a minerotrophic peat bottom
R3 α-mesosaprobic, medium-sized, slightly meandering, slowly flowing small rivers
R4 α-meso-ionic, β-meso- to α-mesosaprobic, linear shaped small to medium-sized waters
R5 α-mesosaprobic, fairly large regulated rivers or stagnant canals

R7 oligo- to α-mesosaprobic, medium to fairly large stagnant canals
R8 β-mesosaprobic, α-meso-ionic, very large, round to irregularly shaped lakes
R9 α-meso-ionic, α-mesosaprobic lower reaches of regulated streams or slightly flowing very small rivers
R11 β-meso- to α-mesosaprobic, α-meso-ionic, mesotrophic, large, linear, slightly flowing rivers or stagnant waters
R12 β-meso- to α-mesosaprobic, meso- to eutrophic, large, less deep stagnant waters

Pools and lakes

P1 temporary, acidified, oligo-ionic, α-meso- to polysaprobic, mesotrophic moorland pools
P2 permanent, acid to acidified, oligo-ionic, α-mesosaprobic to polysaprobic, mesotrophic moorland pools
P3 permanent, slightly acid to acid, oligo-ionic, α-mesosaprobic pools
P4 slightly acid to neutral, α-mesosaprobic, vegetation-rich, small, shallow pools
P5 permanent, α-mesosaprobic, eutrophic, very shallow (swampy), small ditches
P6 clear, well oxygenated, β -mesosapobic, meso- to eutrophic waters (peat pits) with a rich vegetation on a minerotrophic peat bottom
P7 β-mesosaprobic, clear, well oxygenated, meso- to eutrophic, medium-sized, deep stagnant waters rich in vegetation
P8 β-meso- to α-mesosaprobic, medium-sized, stagnant shallow waters
P9 α-mesosaprobic, fairly large ponds or small lakes
P11 β-mesosaprobic, medium-sized, deep stagnant waters

8 Synthesis

Community analysis

The goal of this study was to determine the effect on the development or application of a typology or assessment system of different choices concerning (1) taxonomic adjustment of the data, (2) the data and community variables that are included in the analyses, and (3) the techniques chosen.

The results showed that it is difficult to distinguish macroinvertebrate communities. The analysis of community patterns showed the bias on the results of taxonomic adjustment, the completeness of the data, the techniques used, and the community variables chosen. Changes in different steps of the analyses caused differences in classification or assessment results. There are three main explanations, which are discussed in the next paragraphs:

1. Missing species makes results uncertain;
2. Classification and characterisation of communities is an arbitrary process;
3. Communities do only exist momentarily.

Missing species makes results uncertain

The real size of the species pool in an aquatic ecosystem at a moment is very difficult if not impossible to establish (Lenat & Resh, 2001). First, this is caused by spatial variation; the species composition changes along the vertical, transversal, and in rivers also along the longitudinal profile (Vannote *et al.*, 1980; Ward, 1989). Species live at different scales (Frissell *et al.*, 1986); some are confined to a single habitat while others cover a whole river stretch searching for food in all habitats (Cummins, 1973). Second, seasonal variation plays a role, e.g., a number of species have life stages during which they live outside the water (e.g., adult insects). Third, variation among years is regularly observed (e.g., Resh & Rosenberg, 1989; Moller Pillot, 2003). Because of spatial and temporal variation, it is impossible to determine where one community ends and another starts, thus which area at which scale should be sampled exactly. Boundaries are vague and therefore subjectively chosen by the observer (Armitage, 1961). Fourth, it is impossible to representatively collect all species that are present at a certain moment at a site. Verdonschot (1990) showed that about half of the species actually present was collected using a macroinvertebrate pond net. Vlek (2004) observed that there is a large difference between replicates taken from one site at one day. Each sample contains some information about the community but none is complete (Giraudel and Lek, 2001). This is not a specific macroinvertebrate problem. It was also observed for fishes (Cao *et al.*, 2001) and macrophytes (Dawson *et al.*, 1999).

Community analysis is generally assumed to overcome the sampling problem by comparing species compositions of samples. Samples with similar species compositions are considered to represent the same community. Although samples are incomplete, classification of sites may be possible using the species that are common and/or have relatively high abundances. Classification of samples is based on the assumption that the species that are common and characteristic for a community have relatively high abundances, so their chance to be collected is relatively high (Townsend *et al.*, 2000). Classification techniques merge samples with similar abundances of widely distributed species, while the rarer ones are distributed randomly over the samples in a cluster. Furthermore, in multivariate analyses it is assumed that classifying samples including variation in species composition in one group gives a complete view of the whole community (species that are missed in one sample can be present in another one). The sites together in one group describe the species composition of the community. Species that occur in low numbers or in few samples are randomly scattered over the data. This is a phenomenon of ecological data (Legendre & Legendre, 1998) and generally not considered as a problem. The rare species (which are considered to occur incidentally and therefore would not be characteristic for a community) are often downweighted or excluded from calculations to avoid that they influence the classification (Gauch, 1982; Marchant, 2002).

Although rare species are often missed, they are important in community analyses. The results presented in chapter 4 showed that both species with low abundances and species with small distribution ranges are important in assessing the ecological quality of a site. Species with low abundances could be low abundant because they live in suboptimal conditions and are therefore negative indicators for the community. Chapter 4 and 5 both explained the importance of species with a small or scattered distribution range by showing the relationship between the numbers of these species in a sample, environmental variables indicating unimpacted sites and ecological quality. For biodiversity conservation rare species are important aspect as well. To know which species are vulnerable and need protection of their habitat, their distribution should be known. The absence of rare species in a taxa list of a site might cause a water body not being protected or managed properly although that would be desirable concerning the rare species that actually occur there. Only by species level identifications rare species can be identified and play a role in the analyses.

Missing species because of identification problems and taxonomic adjustment of the data set also results in different classifications as was shown in chapter 2. In fact, the identification of the specimens to genus or higher taxonomic level has a similar effect as missing taxa during collection or excluding a high number of taxa from the data, namely reduction of taxa richness and variation in the data set. Sometimes, this can not be avoided, for example because a specimen is still too small to be able to identify it. Because of reduced richness and taxonomic variation, assessment of the ecological quality can be more difficult (the discrimination between ecological quality

classes of several metrics is reduced as was shown by Schmidt-Kloiber & Nijboer, 2004). What's more, species with different biological traits are merged into one group. Taxonomic adjustment to higher taxonomic levels can result in clustering of samples based on for example some genera, which could have included different species, consequently with different traits and distributions and thus indicating different environments (chapter 2, Schmidt-Kloiber & Nijboer, 2004).

One should always keep in mind that analysing samples that are probably never complete can give results that differ from the results that would have been achieved if the complete species assemblages were collected. This resulted from chapter 6 and 7 in which analysing partial data sets was compared with analysing complete data sets. Incomplete data affect classification, assessment of ecological quality as well as conservation value. Especially, rare species with small distribution ranges showed a relation with ecological quality (chapters 4 and 5). To avoid a high number of rare species not to be collected, the sampling strategy should be carefully chosen according to the scale and the water type concerned. Furthermore, samples collected within one study, should be collected following a standard protocol to make the results between samples comparable. In conclusion, samples should be collected in a similar way, as complete as possible, identification should be to species level if possible, and no taxa should be excluded before analyses.

Community analysis is arbitrary

If as many species as possible are collected, identified and included in the data set it is assumed that the main community pattern can be described. Multivariate techniques have been used to understand ecological data, to extract communities, and to relate these to the environment (e.g., Gauch, 1982; Jongman *et al.*, 1995; Ludwig and Reynolds, 1988; Legendre and Legendre, 1998). Several classification techniques can be used to group the sites using their species composition, such as clustering techniques (e.g., Hill 1979, Van Tongeren, 1986) or the recently developed artificial neural networks (Lek & Guegan, 2000). Techniques are supposed to clarify and give insight in the community structure using similarities and dissimilarities between the species composition of sites. Analysing a data set with two techniques should result in similar classifications including a similar number of community types (site groups). The optimal number of site groups is achieved if the most appropriate similarity level is achieved (when the differences among site groups are maximal and differences between samples within site groups are minimal) (Usseglio-Polatera *et al.*, 2000). Those rules often are based on the idea of a hierarchy in which few large groups are split in smaller groups going down a similarity dendrogram.

That techniques do not always give similar results was shown in chapter 6, where two techniques were compared and resulted in classifications with only about 50% overlap. This was probably caused by the community variables the techniques focus

on. The choice of the community variable is especially of importance if the variation within the data set is small (Giraudel & Lek, 2001). Probably, the community structures in the data used in chapter 6 were not apparent enough to overcome technical differences between both methods used. Samples can differ using one community variable, but be similar using another variable. Only the most extreme samples (e.g., fast flowing streams or brackish channels were classified similarly by both techniques (chapter 6), probably because they show differences for a high number of community variables.

Also, choosing the optimal number of site groups appeared to be difficult (chapter 6). Isolation values (similarity within site groups/similarity between site groups) were not different using different numbers of site groups. Reducing the number of site groups did not result in merging of small clusters into large ones. Instead, new site groups with completely new combinations of sites were constructed. This indicates that hierarchy is missing in both methods used. Such tendencies make it difficult to evaluate and interpret the results of a classification. Many different stopping rules have been proposed to determine the optimum number of site groups that can be recognised in a certain dendrogram (review: Milligan & Cooper, 1985).

In conclusion, many choices influence the classification results which makes it a subjective process. Analysing large data sets is therefore always an interpretation of the real situation; it is difficult to make the complete community structure apparent (Giraudel and Lek, 2001). The choice of the classification method is crucial. It is important to fully understand the properties of the methods used to be able to interpret the results (Legendre & Legendre, 1998) and to choose the method that best fits with the objectives of the study. This choice is especially important if the gradients in the data are not clear (chapter 6). Giraudel & Lek (2001) also found that if a data set is small and shows clear gradients, different techniques show similar results, but if the gradients in the data are less clear, different techniques might result in different community structures. The optimal number of site groups can not be determined using similarity criteria; instead practical criteria are often used. Furse (2000), for example, stopped the division of site groups when any daughter group contained less than five sites.

Communities do only exist momentarily

The thought behind community analysis is that within a restricted geographic area the species assemblages at two sites with similar environmental conditions will be similar and both represent the same community. Clements (1916) regarded the community as an association, of which the whole is more than the sum of its parts. The distribution and abundance of a species in a community are determined by its interaction with other species in the same community. Species making up the community typically are associated with each other and are organised into discrete groups.

Contrary to the concept of a community being a strict association between species, general observations confirm that species comprising a community do not necessarily associate exclusively with one another. Rather, each species appears to be distributed in its own way, according to its own response to varying environmental conditions such as altitude, temperature, nutrients, and other physical conditions, the individualistic species approach (Gleason, 1926; Ramensky, 1926). Biological conditions are also relevant: the ability to colonise, grow, and to overcome competition and predation pressure. Biotic interactions are not necessarily confined to specific other species. Often they relate to a certain function, which can be represented by different species. Overall, chance is heavily involved. Therefore, two sites with similar environmental conditions can be inhabited by different species assemblages as was observed by Moller Pillot (2003) for dynamic stream macroinvertebrate communities. Species did in this approach not react as community members but as units that were independent from each other. As such, communities within a restricted geographic area are spatially unique and in time momentary assemblages of species (Hengeveld & Bijlsma, 1995).

Some species will succeed only in certain environmental situations and tend to be confined to certain habitats. They have a restricted distribution along an environmental gradient. Others are more tolerant and occupy a wider distribution along an environmental gradient. This sequence of communities showing a gradual change in composition is called a continuum (Whittaker, 1952; Curtis, 1959). Each community is somewhat different from its neighbour, the difference increasing roughly as the environmental distance between them increases.

That communities can not easily and unambiguously be characterised does not only depend on the techniques used and the choices made. If the continuum concept is combined with the stochastic aspects (the chance of colonisation) and biological traits and interactions of species, the differences between communities that are easy or difficult to distinguish, can be explained. Communities that are easily recognised by different techniques are communities that are on the endpoints of a continuum, where values of certain environmental variables are extremely high or low (Chapter 6). Chapter 7 showed that communities with a large number of indicator species were the communities with more extreme environmental conditions. Assigning samples of these communities to a typology resulted in fewer errors than for communities with few indicative species. These environmental conditions have a natural origin, e.g., acid conditions or a high salinity and are considered as unfavourable (Southwood, 1977, Fig. 1). Species have developed specific adaptations to these habitats during a long evolutionary time period. These are adversity-selected species (A-selected species, Southwood, 1977). Many A-selected species have limited distribution ranges, only surviving as long as their habitats exist (Greenslade, 1983). Therefore the number of rare species in these habitats is high. The species richness of sites with extreme environmental conditions is low and the species present have broad niches

(Southwood, 1977). There are clear relationships between the species present and the environmental constraints. Adaptations to the environment cost energy and there is always a trade-off between biological traits (e.g., Townsend *et al.*, 2000). If a species has invested in adaptations to overcome physical problems, there is less energy left for e.g., maintenance and growth. The competitive capacity of A-selected species is often also low, except for their own special habitat in which they can efficiently use the resources available (Pianka, 1978). That is why they are absent in more favourable environments where they come off worst. Species without specific adaptations, i.e., generalists can occur in unfavourable conditions, but they live under suboptimal conditions and therefore, their abundances are low.

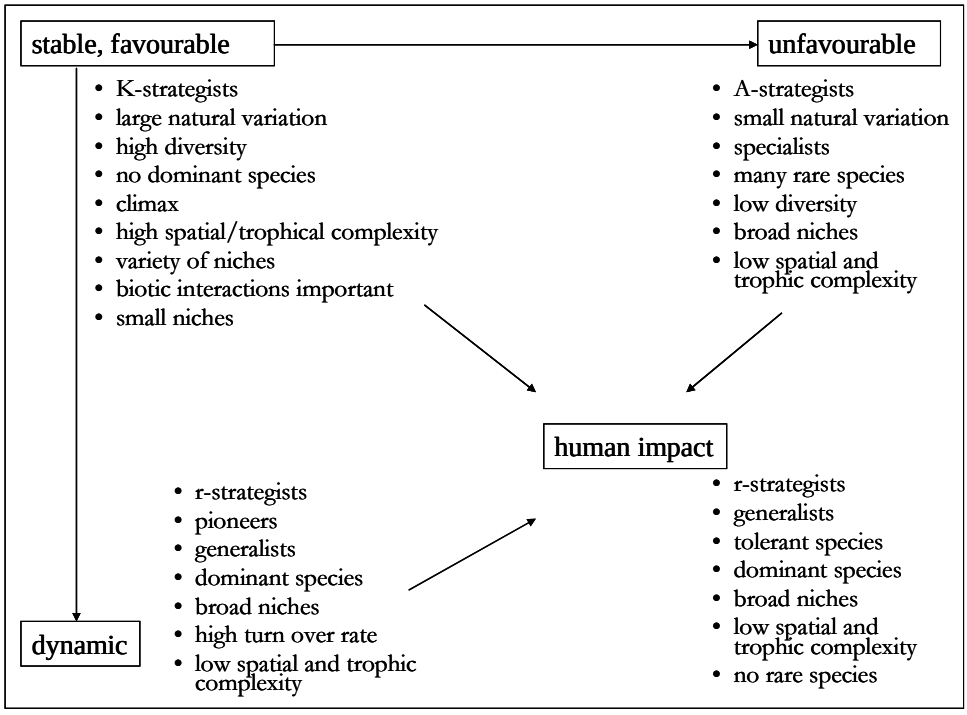

Fig. 1. The characterisation of macroinvertebrate communities along two axes, one from dynamic to stable environments and one from favourable to unfavourable (adverse) environments. Human impact can result in more dynamic and/or unfavourable conditions (modified after Southwood, 1977).

In more favourable environmental circumstances (Fig. 1), many more species are able to survive even if they live under suboptimal conditions. The environment is more complex and species have smaller niches. Their presence does not particularly depend on the environmental conditions. Specific adaptations to environmental

extremes are not needed. Under these conditions biotic interactions such as competition, predation and defence against predation, and the chance that a species can colonise the water body often play a more important role. Species with specific adaptations are out competed in these environments. Common species occur in these communities but none of the species is really dominant. Species in this type of environment are often K-strategists, species that allocate more energy to growth and maintenance (MacArthur & Wilson, 1967) instead of reproduction. Because there is variation between the species composition of environmentally similar sites, the distinction between natural variation and variation caused by human impact can be difficult to make as long as the impact factor is not too strong.

If the environment is more dynamic, for example a stream with a variable discharge regime, including peaks and periods in which the stream dries up, r-strategists take over. R-strategists allocate more energy to reproduction and a high level of dispersal (Southwood, 1977). The dynamic environment continuously creates new habitats, which are inhabited by pioneers, the production is high and because the number of species is still low, the species have broad niches. The diversity depends on the frequency of disturbance. Following the intermediate disturbance hypothesis (Ward & Stanford, 1983) the most dynamic habitats have low diversity. Diversity is increasing at intermediate disturbance, because new habitats are created but decreases again if the environment becomes more stable.

Human impact can cause a more dynamic environment (e.g., by causing discharge peaks in a stream due to drainage of the catchment area) or a less favourable environment (e.g., oxygen depletion due to organic pollution). If the environment becomes more dynamic, the gradient to a dynamic ecosystem can be followed and it will be characterised by r-strategists and pioneers. Taxa richness will first increase (chapter 5) but if the system becomes too dynamic decrease again. The system becomes less complex concerning trophic relations and other biotic interactions. If human impact causes an unfavourable environment, first the relative abundances of the species will change, tolerant species becoming more dominant and more sensitive species decreasing in numbers of individuals, because less offspring will survive or because they are out competed by the more tolerant species. Because environmental degradation is a fast process in comparison to evolutionary changes, species are not able to develop specific adaptations to these conditions. Therefore these unfavourable environments are not characterised by A-selected species. Species that can survive environmental degradation are generalists. They have biological traits that enable them to overcome different problems. Their trade-off is probably that less energy is spend at competition. That is why there numbers are restricted in stable, favourable environments. If other species disappear the generalists can become dominant. This also explains that different types of human impact causing unfavourable result in similar species compositions (Verdonschot, 1983).

It can be concluded that communities do not exist in such a way that species assemblages occurring at different sites with similar environmental conditions can be regarded as the same community. A community can only be defined as a combination of species populations occurring at a certain site at a certain moment. Communities can be characterised by their ratio between A, r, and K-selected species and the biological traits that belong to these strategies and related to the stability and favourableness of the environment at a site. Missing species because of sampling, sorting and identification problems and subjective choices that have to be made in analysing techniques enlarge the difficulties to clearly describe a community. In unfavourable environments characterised by species that are specifically adapted to environmental conditions, communities are more constant and recognisable than under moderate conditions, where coincidence and biotic interactions play a major role. The latter communities are the most difficult ones to describe because of their large natural variation in space and time. In these situations methodological choices and the completeness of samples have the largest influence on the resulting typology or assessment system. If the environment is impacted by human activities, generalists that are relatively tolerant become dominant and sensitive species disappear because of the environmental constraints or because they loose competition from the generalists. Different types of environmental degradation are characterised by similar communities of few species, which have high abundances.

From structure to function

From the previous paragraph can be concluded that strategies and biological traits might be important to understand the species composition in relation to the environmental conditions of a site. This approach might have advantages above a species approach. Siepel (1994a) stated that due to the enormous species diversity of microarthropods, with the many different ways these species are affected by human influences, it is often impossible to extract clear patterns from a species list, which was underlined by the present study. The majority of community structure studies is based on correlations and gives no grip on possible causal mechanisms (Siepel, 1994a, Jongman *et al.*, 1995). This also applies to aquatic macroinvertebrates (Wright, 2000), because this group also consists of a large number of species with many different biological traits. Biological traits are assumed to reflect adaptations to dominant environmental characteristics and/or stresses of habitats, and therefore have the potential to give a better understanding of the mechanisms structuring communities (Southwood, 1977, Townsend & Hildrew, 1994; Statzner *et al.*, 1997). Non-taxonomic aggregations of taxa into trait categories might be more effective for investigating mechanisms affecting species distributions for assessing environmental conditions, and for guiding management (Grime, 1997, Richards *et al.*, 1997, Statzner *et al.*, 1997).

From earlier applications appeared that individual traits may serve to bio monitor different types of human impact (Dolédec *et al.*, 1999). In freshwater macro-invertebrate trait studies a distinction is made between biological traits (such as reproduction or respiration features) and ecological traits (which are in fact ecological preferences, e.g., for current velocity, acidity, et cetera). Dolédec *et al.* (1999) observed that biological traits were more useful to indicate human impact in running waters than ecological preferences. This could be explained by the direct relation between biological traits and the ability to survive under the prevailing environmental conditions.

There are some problems with biological trait analyses. First, there is the choice which traits should be included. Dolédec *et al.* (1999) used 15 biological traits, while Usseglio-Polatera *et al.* (2000) used 11 biological traits. It is important not to miss relevant traits but sometimes this is inevitable because information is lacking. Usseglio-Polatera *et al.* (2000) had to omit e.g., fecundity and dispersal potential. On the other hand, traits that are not relevant might be included, e.g., food as it was used in Usseglio-Polatera *et al.*, (2000) and Charvet *et al.* (2000). This 'trait' can be dependent on the food sources available (Becker, 1990, 1994). The fact that a species can change from one to another source or has an ontogenetic shift would be more useful as a trait because it reflects flexibility thus an adaptation to a changing environment.

Second, taxonomic resolution is a major discussion point in species trait analyses. In many species trait studies, traits are not linked to species but to genera or families (e.g., Charvet *et al.* 2000). Pianka (1978) stated that physical mechanisms (biological traits) clearly must reflect ecological conditions. This implies that ecological preferences are related to biological traits. Ecological preferences within a genus can be quite different (e.g., Graf *et al.*, 1995). Although less is known for biological traits, there are numbers of examples of differences between species within a genus documented in literature. For example, species within the genus *Gammarus* have different life spans and different numbers of generations per year (Gledhill *et al.*, 1993). Their different salinity tolerance (Gledhill *et al.*, 1993), an ecological preference, suggests that there are biological (physiological) traits that differ between the species. Merging species into genera and then assigning the affinity to trait categories to the genus results in errors for biological traits.

Third, the division in of traits in categories influences the results. Traits are not discrete (Siepel, 1994b) and species can not always be put into one category (Chevenet *et al.*, 1994). Therefore, Usseglio-Polatera *et al.* (2000) used a score from 1 to 5 to each taxon describing its affinity to each trait category. This might better represent the reality but it enlarges the fourth problem, which is the classification of traits.

Fourth, trait classification might be arbitrary. Smith (1986) stated that the species is the only unit that has a clear relation with the environment and with other species. Species respond independently to the biotic environment according to their own

genetic characteristics. Pianka (1978) explains this by the statement that 'Any given organism has a unique co-adapted complex of physiological, behavioural and ecological traits, whose functions complement one another and enhance that organism's reproductive success'. This is called an optimal design (Rosen, 1967), an adaptive suite (Bartholomew, 1972), or a tactic (Stearns, 1976). Tactics are useful in analysing the effect of nature management and of the pollution of biotopes (Grime *et al.*, 1988). But, if each organism or each species has a unique combination of traits, classification of species with similar tactics is artificial and the results will be influenced by the techniques used and the choices made within these techniques. This was shown by Usseglio-Polatera *et al.* (2000) who defined 8 groups and 15 subgroups depending on the similarity level used to split the trait groups. Siepel (1994b) stated that the number of tactics is limited. He defined 12 tactics for soil microarthropods (1994b). Many traits are spin-offs (Pianka, 1978) or trade offs (Siepel, 1994a) of other traits and related to each other and to the general body plan of the organism (Pianka, 1978). Therefore, not each theoretical combination of traits would really occur. This implies that a large number of species share the same tactic. This concept raises the question why species with the same tactic show different responses and sensitivity to changes in their environment. Small changes along an environmental gradient do not result in the simultaneous disappearance of all species with a certain tactic. Some species are more sensitive than others and the question is why? Probably, this is due to the fact that traits are not discrete, and the fact that each species has a different general body plan, which determines the energy needed to maintain a certain tactic. The trade-off of needing more energy to maintain a tactic can result in less competitive strength. Thus, to detect small changes a species composition analyses is still necessary. This is relevant for an early detection of human impact and to be able to restore the required environment as soon as possible and to avoid species to disappear.

Species traits can be useful, at least if specimens are identified to species level and then assigned to trait categories. Biological traits should be used instead of ecological preferences to be able to explain the ability of a species to survive under the prevailing environmental conditions. However, to detect small environmental changes a species composition analysis might be inevitable; especially if not all relevant biological traits are known yet. Vulnerable species (species that have low abundances and a small distribution range and are declining) are not directly observed if they have no specific tactic. For conservation purposes it is useful to analyse which species are rare and which tactics they have. Knowing the relation between tactics and ecological conditions of rare species is important for their conservation through restoring the environmental conditions they require (Nijboer, 2004). Knowing the tactics of rare species can give a clue whether they are rare because they live under natural but extreme conditions (they have specific adaptations) or because their habitat has been degraded by human impact (no specific adaptations). This underlines the importance

to continue studies on tactics of species to improve our knowledge (Usher *et al.*, 1982; Usseglio-Polatera *et al.*, 2000). Knowledge about biological traits is not only necessary for analyses to relate tactics to environmental conditions but also for interpretation of community analyses based on species distributions. For analysing tactics of aquatic macroinvertebrates, the first step would be to select the relevant traits and define the categories. To test information from literature and correlative data analyses an experimental design in the laboratory and in the field should be used to study which tactics species have and what the relations between tactics and environmental conditions are. Besides, such experiments are useful to detect the species traits that are most relevant to survive in a certain environment. Finally, to understand minor shifts in species composition, the reason why species with the same tactic have different responses should be studied.

Implications for ecological assessment and conservation of biodiversity

As was stated in the introduction, assessment of the ecological quality of a surface water and conservation of biodiversity are two different premises in protection and restoration of habitats and water bodies. From this study appeared that analysing large data sets to reveal community patterns and to develop assessment systems is arbitrary. Therefore, this should not be used unless really necessary. Assessment using a typology and an assessment system based on a large data set is only necessary if quality measurements between surface waters should be compared, as is the case for the Water Framework Directive (European Commission, 2000). The subjective process of developing and applying an assessment system makes lots of escapes from the criteria in this Directive possible. The assessment system and sampling strategy mainly determine the ecological quality that is measured. For a strict use of the Water Framework Directive standardisation of the whole process is crucial (Hering *et al.*, 2004). However, this is a difficult task because each country and each water board has its own methods, developed during a long history of water management. An assessment system should focus on the combination of dominant species, indicator species and the number of rare species. Metrics, related to tactics of these species are probably most successful.

For regional water management precise assessment of the ecological quality is unnecessary. Water managers need to identify stressors on the one hand and requirements of species on the other hand to steer restoration in the right direction (Verdonschot & Nijboer, 2000, Verdonschot & Nijboer, 2002). Monitoring of the ecosystem at a site is needed to spot degradation or effects of restoration, if possible by comparing data with taxa lists of reference conditions (Verdonschot, 1990). Because each location is different and has its specific features and problems, a site

specific approach is more useful than analysing large data sets of different sites. For assessing the ecological conditions of a site, in which relations between species composition and human impact are of importance, it is important to link the tactics of the species to the stability and favourableness of the environment. The complete taxa list is relevant. Grouping the species found at the site according to their tactics gives insight in which tactics are lacking, which ones occur in low numbers and which ones are dominant. This can give clues about the environmental conditions.

Conservation of biodiversity should focus on rare species and habitats as was explained in the introduction. Taxa richness of a sample indeed did not relate to special habitats or high ecological quality and is therefore not useful in assessment of ecological quality or conservation value. In contrast, the number of rare taxa (with small distribution ranges) can indicate the importance of a site for conservation of biodiversity. Because the number of rare species is related to specific habitats or ecological quality, rare species are important for both purposes. Conservation of biodiversity focuses except for rarity also on the trend of a species. Species that are rare and show a declining trend are vulnerable and probably indicative for degradation. Again, knowledge of tactics of these species is important to interpret their presence or absence and to determine the (environmental) variable that causes a decline. Indicator species are useful for conservation purposes because they can indicate specific habitats where rare species might occur. This role is important although indicator species are not of direct importance for conservation of biodiversity.

In conclusion, ecological assessment, regional water management and conservation of biodiversity should focus on tactics of all species observed at a site in relation to the stability and favourableness of the environment to be able to diagnose human impact and to restore a water body successfully. Basically, each species or each tactic is a potential candidate to indicate human impact. Identification to species level is always needed because tactics differ between species within a genus and to identify rare species.

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Summary

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Macroinvertebrate community patterns are often analysed and related to the ecological quality or conservation value of a water body, using an ecological typology or assessment system. There is not one general methodology to develop or apply such a tool. There are many choices to be made, based on either scientific or efficiency criteria. The goal of this study was to determine the effect of (1) taxonomic adjustment of the data, (2) taxa and community variable selection, and (3) the techniques chosen, on the development and application of a typology or assessment system.

The first step in developing a typology or assessment system is the taxonomic adjustment of the data. In the ideal situation a typology or assessment system is based on species data to include as much information as possible and to make adjustment unnecessary. However, this is not always possible. Data that include different taxonomic levels have to be made consistent throughout samples to make them comparable and useful for analyses. However, different methods of taxonomic adjustment (adding abundances of species at a higher taxonomic level or deleting higher taxonomic levels) resulted in different classification of samples (chapter 2), which finally affects the development of a typology or assessment system.

The second choice that has to be made is which community variable(s) will be used in the analyses. A data set with macroinvertebrate samples is characterised by the distribution and abundance of the species (common versus rare species) among the samples, the total number of species in a sample, and the indicativeness of species for groups of samples.

Including rare species if often subject of discussion. The number of species with small distribution ranges in a sample was positively related to high ecological quality of streams (chapter 4 and 5). The number of species with small distribution ranges was positively related with environmental variables indicating naturalness and negatively to variables indicating disturbance. But, there was also a relationship with stream or channel characteristics, such as a high current velocity. This indicates that species with small distribution ranges can indicate specific environmental conditions. Although there are only few species with small distribution ranges in a sample, this number is still useful to indicate conservation value and ecological quality (chapter 5).

To avoid noise, species with low abundances or species occurring in few samples are often excluded from the analyses. However, chapter 4 showed that in both cases

this resulted in other ecological quality classes using the AQEM assessment system. If taxa with small distribution ranges were excluded, the samples were classified into a lower ecological quality class, because many of the taxa with small distribution ranges indicated special habitat conditions or unimpacted sites. Although taxa with low abundance in a sample seemed not relevant, ecological assessment of the samples without these taxa resulted in a higher ecological quality class, because non-indicative taxa or taxa not characteristic for streams were deleted. Also, classification of samples excluding taxa with low abundances resulted in completely different outcomes using two different classification techniques (chapter 6).

Taxa richness of a site is often used to indicate human impact. However, the results presented in chapter 5 showed that this is not possible for Dutch streams and channels. The total number of taxa in a sample was not related to environmental variables indicating a high or low ecological quality. Taxa richness rather depended on typological differences. Excluding typological differences resulted in optimum curves for taxa richness in streams and channels. Increasing degradation first resulted in an increase in taxa richness before taxa richness decreased. Therefore, it can be concluded that taxa richness is not suitable to estimate ecological quality or conservation value.

Indicator species are another variable that can be used to identify ecological quality. Unfortunately, indicator species can only be detected after the whole data set is analysed, because the complete data are necessary to conclude which species are indicative for a community. But, as was shown in chapter 6, if a classification is repeated with only indicator species, this results in a completely different classification. This implies that applying a typology by only including indicator species will neither be a success. This was confirmed by the results in chapter 7, which showed that assigning a sample to a typology using only indicator species did not work. Another attempt to reduce the data by using species from one taxonomic group also showed many misclassifications (chapter 7) in reassigning samples to a typology.

Third, analysing community patterns asks for mathematical techniques that are able to show differences and similarities between samples. Ideally, different techniques should result in similar classifications. However, from chapter 6 appeared that this is not the case at all. Analysing community patterns appeared to be difficult and not objective. Fifty percent of the sites were clustered with other sites if non-hierarchical clustering was used instead of a self organising map or the other way around. Differences depend on the community variables on which the technique focuses. One technique is not always better than another; one should therefore choose the most appropriate technique depending on the goal of the study and the application of the typology.

Also within techniques the choices made influence the final results. The most important choice is the number of types that is allowed in a typology. However, it is

not possible to determine the optimal number of types. There were no differences in distinction between types in a fine and a coarse classification using 'isolation value' (similarity between samples in a type divided by similarity between the type and the most similar type) as a measure (chapter 6). Therefore, it might be better to relate the number of clusters to the goal of the classification.

Relating environmental variables to macroinvertebrate samples using a Self Organising Map and Canonical Correspondence Analysis showed only small differences between techniques. Differences only occurred for environmental variables of minor importance. The main gradients in the data were identified with both techniques (chapter 6).

In conclusion, it is very difficult to distinguish and characterise macroinvertebrate communities unambiguously. An additional problem concerns the sampling. It is not possible to representatively sample all species present at a certain moment at a site. Only a part of the community is represented in a sample, which influences the results. The analysis of community patterns showed the importance of taxonomic resolution and adjustment, the completeness of the data, the variables chosen, and the techniques used. Therefore, community analysis is not an objective process. Besides, communities do not exist in such a way that species assemblages occurring at different sites can be regarded to be the same community because of a number of overlapping species that occur under similar environmental conditions. A community can only be defined as a combination of populations occurring at a certain site at a certain moment. The sequence of species assemblages on an environmental gradient is a continuum. In unfavourable environments species assemblages are more constant and include more A selected and rare species with specific adaptations to the environment. In favourable environments, where biotic interactions play a major role the majority of species is K selected. Natural variation is larger in these environments. In dynamic or impaired environments generalists (r selected species) take over and can reach high abundances. Especially in favourable environments where natural variation is large methodological choices can have high influence on the resulting typology or assessment system.

Because analysing large data sets to reveal community patterns and to develop assessment systems is quite arbitrary, analysing large data sets is always an interpretation of the real situation. Therefore, one should avoid analysing large data sets by extracting community patterns, unless necessary because the quality between many sites has to be compared. For regional water management, precise assessment of the ecological quality is unnecessary. But, water managers need to identify stressors on the one hand and requirements of species on the other hand for successful restoration and management. Monitoring of the species and their abundances within an ecosystem is needed to spot causes of degradation or effects of restoration. Because each location is different, the species list of a single site or water body should be

analysed and interpreted. Life tactics of species can be related to the habitat templet of favourability, impairment and stability and thus be used to diagnose the stressor in a water body. For assessing the conservation value the number of rare species and their life tactics is important. Additionally, indicator species can give clues about special environmental conditions. For both purposes, identifying the ecological conditions and assessing the conservation value, knowledge about the species' tactics should be extended to be able to interpret the results. Therefore, experimental research to extract and explain relations between species, their tactics, and environmental variables is of great importance.

Samenvatting

Kenmerken van macrofaunalevensgemeenschappen worden vaak gebruikt voor het bepalen van de ecologische kwaliteit of de natuurwaarde van oppervlaktewateren. Hiervoor worden de levensgemeenschappen geanalyseerd en opgenomen in een typologie of beoordelingssysteem. Er zijn vele mogelijkheden voor de ontwikkeling van dergelijke instrumenten. Binnen iedere stap in de procedure van bemonstering tot beoordelingssysteem moeten keuzen gemaakt worden. Hiervoor worden niet alleen wetenschappelijke maar ook economische criteria gebruikt. Het doel van dit onderzoek was het bepalen van het effect van (1) taxonomische afstemming van de data, (2) selectie van taxa en levensgemeenschapsvariabelen en (3) de gekozen technieken op de ontwikkeling en toepassing van een typologie of beoordelingssysteem.

De eerste stap in de ontwikkeling van een typologie of beoordelingssysteem is de taxonomische afstemming van de data. In het ideale geval zijn alle dieren tot soort gedetermineerd, zodat de informatie zo volledig mogelijk is en taxonomische afstemming niet nodig is. Dit is echter zelden het geval. Data waarin overlap plaatsvindt tussen taxonomische niveaus moeten consistent gemaakt worden, zodat de monsters vergelijkbaar zijn en als één dataset geanalyseerd kunnen worden. Er zijn verschillende mogelijkheden voor taxonomische afstemming maar iedere methode leidt tot andere resultaten in de classificatie van de monsters (hoofdstuk 2). Dit bepaalt het uiteindelijke resultaat van een typologie of beoordelingssysteem.

Vervolgens moet gekozen worden op welke levensgemeenschapskenmerken de nadruk gelegd wordt. Een macrofauna dataset wordt gekenmerkt door de verspreiding en abundantie van de taxa (algemene versus zeldzame taxa) over de monsters, het totale aantal taxa in een monster en de indicatieve waarde van de taxa voor groepen van monsters.

Het al dan niet meenemen van zeldzame soorten in de analyses is vaak onderwerp van discussie. De hoofdstukken 4 en 5 hebben echter aangetoond dat zeldzame soorten juist zeer bruikbaar zijn. Het aantal soorten in een monster met een smalle verspreidingsrange in Nederland was positief gerelateerd aan een hoge ecologische kwaliteit in beken (hoofdstuk 4). Tevens bleek er een positieve relatie te zijn tussen het aantal soorten met een smalle verspreidingsrange in een monster en milieuvariabelen die natuurlijkheid indiceren en een negatieve relatie met variabelen die duiden op verstoring (hoofdstuk 5). Maar ook bepaalde karakteristieken van beken of sloten die losstaan van menselijke beïnvloeding waren gerelateerd aan een hoog aantal van deze soorten, zoals een hoge stroomsnelheid in beken. Dit betekent dat soorten met een smalle verspreidingsrange ook bijzondere milieus kunnen indiceren. Ondanks het feit dat het aantal van deze soorten in een monster meestal laag is, is dit

aantal goed bruikbaar om ecologische kwaliteit of natuurwaarde te indiceren (hoofdstuk 5).

Om ruis in de data te voorkomen worden soorten met een lage abundantie of soorten die een lage frequentie hebben vaak verwijderd. In hoofdstuk 4 is echter aangetoond dat dit voor het AQEM beoordelingssysteem resulteert in een andere ecologische kwaliteitsklasse. Als taxa die een smalle verspreidingsrange hebben in Nederland worden verwijderd, leidt dit tot een lagere ecologische kwaliteitsklasse, omdat veel van deze soorten onbeïnvloede wateren of bijzondere omstandigheden indiceren. Daarentegen leidt het weglaten van soorten met een lage abundantie in een monster tot een hogere ecologische kwaliteitsklasse, doordat deze taxa niet indicatief zijn voor een goede kwaliteit of juist indicatief zijn voor een slechte kwaliteit en/of omdat deze soorten niet karakteristiek zijn voor het beekmilieu. Ook classificatie van monsters waarbij de soorten met de laagste abundanties zijn weggelaten leidde tot een geheel andere resultaat dan wanneer de volledige dataset werd gebruikt. Dit gold voor twee verschillende technieken (hoofdstuk 6).

Het aantal taxa in een monster wordt vaak gebruikt om menselijke beïnvloeding te indiceren. In hoofdstuk 5 is echter gebleken dat dit niet mogelijk is voor de Nederlandse sloten en beken. Het totale aantal taxa in een monster bleek niet gerelateerd te zijn aan milieuvariabelen die een lage of hoge ecologische kwaliteit indiceren. De taxarijkdom was meer gerelateerd aan typologische kenmerken van de wateren. Daarom kan geconcludeerd worden dat het aantal taxa in een monster geen goede variabele is om te gebruiken voor het bepalen van de ecologische kwaliteit of de natuurwaarde van een oppervlaktewater.

Soorten die indicatief zijn voor een levensgemeenschap in een waterlichaam zouden goed bruikbaar moeten zijn voor beoordeling van ecologische kwaliteit en natuurwaarde. Een nadeel is dat eerst alle data geanalyseerd moeten worden om te bepalen welke taxa indicatief zijn. Na deze eerste analyse zou een tweede analyse met de indicatieve soorten tot vergelijkbare resultaten moeten leiden. Dat dit niet het geval is, is gebleken in hoofdstuk 6 waarin het gebruiken van indicatieve soorten leidde tot een geheel andere classificatie van de monsters. Hetzelfde geldt voor de toepassing van een typologie (hoofdstuk 7). Het gebruiken van slechts de indicatieve soorten voor het toedelen van een monster aan een bestaande typologie resulteerde in een hoog percentage foute toedelingen. Hetzelfde gold voor het toedelen van een monster waarbij alleen de taxa uit één taxonomische groep gebruikt werden (hoofdstuk 7).

Een derde belangrijke factor in het ontwikkelen van een typologie of beoordelingssysteem is de keuze van de techniek voor de analyse van de data. Rekentechnieken zouden verschillen en overeenkomsten tussen monsters moeten aangeven. In het ideale geval zouden verschillende technieken tot dezelfde resultaten moeten leiden. Uit hoofdstuk 6 volgt echter dat dit geheel niet het geval is. Het analyseren van macrofauna gemeenschappen blijkt moeilijk en niet objectief te zijn.

De resultaten zijn afhankelijk van de gekozen techniek en de keuzen die gemaakt worden binnen een techniek. De helft van de monsters werd anders ingedeeld als een 'Self Organising Map' werd gebruikt in plaats van 'Non Hierarchical Clustering' en andersom. Verschillen tussen technieken worden veroorzaakt door de karakteristieken van de gemeenschap waar de techniek zich op richt en die vertaald zijn in de onderliggende algoritmen. Meestal is het moeilijk te beoordelen welke techniek het beste is. De keuze van een techniek moet daarom genomen worden op basis van de doelstelling van de analyse en de toepassing van de typologie of het beoordelingssysteem.

Binnen de classificatietechnieken is het aantal monstergroepen dat gevormd moet worden de belangrijkste keuze. Het is echter niet te bepalen wat het optimale aantal clusters is. Gebruik makend van de 'isolatiewaarde' (de mate van overeenkomst tussen de monsters in een groep gedeeld door de overeenkomst tussen de groep en de meest gelijkende groep) kon geen verschil in de sterkte van een fijne en een grove classificatie gevonden worden (hoofdstuk 6). Daarom kan ook het aantal groepen beter afgestemd worden op het gebruiksdoel dan op wetenschappelijke criteria.

Bij het relateren van milieuvariabelen aan macrofaunamonsters met een 'Self Organising Map' en met 'Canonical Correspondence Analysis' waren de verschillen tussen de technieken minder groot dan bij classificatie. Verschillen traden alleen op voor milieuvariabelen die van minder belang waren. De belangrijkste gradiënten in de data kwamen met beide technieken naar voren.

Concluderend kan gesteld worden dat het zeer moeilijk is om patronen in macrofaunalevensgemeenschappen eenduidig te analyseren. Het is niet mogelijk om alle soorten die aanwezig zijn op een locatie representatief te bemonsteren. Slechts een deel zal in het monster voorkomen, wat de resultaten behoorlijk kan beïnvloeden. De analyses in dit proefschrift hebben laten zien dat taxonomische afstemming en resolutie, de volledigheid van de data, de gekozen variabelen en de gekozen technieken allemaal invloed hebben op het uiteindelijke resultaat. Het analyseren van levensgemeenschappen is daarom geen objectief proces.

Daarnaast is het zo dat levensgemeenschappen niet beschouwd kunnen worden als vaste combinaties van soorten die op verschillende locaties met dezelfde milieu-omstandigheden voorkomen. Een levensgemeenschap kan alleen gedefinieerd worden als een combinatie van populaties van soorten die op één moment op één locatie voorkomt. Kleine veranderingen in combinaties van soorten treden op door veranderingen in de milieu-omstandigheden en vormen zo een continuüm langs een abiotische gradient. In ongunstige milieu-omstandigheden zijn soortencombinaties relatief constant en bevatten ze meer soorten die zich specifiek hebben aangepast aan het milieu. Vaak komen veel A geselecteerde soorten voor en het aantal indicatorsoorten en zeldzame soorten is hoog. In gunstige omstandigheden spelen biotische interacties een grote rol en het grootste deel van de soorten is

K geselecteerd. De natuurlijke variatie in dergelijke milieus is hoog. In dynamische of beïnvloede milieus nemen generalisten (r geselecteerde soorten) in aantallen en abundanties toe. Vooral als de omstandigheden gunstig zijn en biotische interacties ervoor zorgen dat de natuurlijke variatie groot is, hebben methodologische keuzes grote invloed op de resulterende typologie of het beoordelingssysteem.

Het analyseren van macrofaunalevensgemeenschappen met behulp van grote datasets is dus arbitrair. Dergelijke analyses zijn altijd een interpretatie van de werkelijkheid. Daarom zou het gebruik ervan beperkt moeten worden tot die gevallen waarin daadwerkelijk een vergelijking van de kwaliteit van een groot aantal wateren nodig is. Voor het regionale waterbeheer is dit zelden het geval. Een precieze beoordeling van de ecologische kwaliteit is niet noodzakelijk. De doelstelling van het waterbeheer is meestal het instandhouden of verbeteren van de ecologische kwaliteit van een oppervlaktewater. Hiervoor is het nodig om te weten welke milieuvariabelen stressoren zijn en wat de vereisten zijn van de soorten die in het oppervlaktewater thuishoren. Monitoring van de soorten en hun abundanties in een ecosysteem is nodig om de oorzaken van verstoring of de effecten van restauratie te bepalen. Maar omdat ieder waterlichaam uniek is, moet ook de soortenlijst van ieder waterlichaam afzonderlijk geanalyseerd en geïnterpreteerd worden. Levensstrategieën van soorten kunnen worden gerelateerd aan de stabiliteit, de mate van beïnvloeding en de geschiktheid van het milieu. Daardoor kunnen ze gebruikt worden voor het bepalen van de stressor en de mogelijkheden voor herstel. Om de natuurwaarde te bepalen is het aantal zeldzame soorten en hun levensstrategie van belang. Indicatorsoorten zijn niet direct van belang in het natuurbeleid maar ze kunnen wel aangeven waar bijzondere milieus voorkomen en waarschijnlijk zeldzame soorten gevonden kunnen worden. Voor beide doeleinden, het bepalen van de ecologische kwaliteit en de natuurwaarde, is kennis over de levensstrategieën van de soorten onontbeerlijk. Daarom is het uitvoeren van experimenteel onderzoek waarin relaties tussen soorten, hun levensstrategieën en het milieu waarin ze voor kunnen komen, worden onderzocht van groot belang.

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Curriculum vitae

Op 11 april 1972 werd ik geboren in Delft. Al snel verhuisden we naar Geldrop waar ik de rest van mijn jeugd heb doorgebracht en het VWO heb gedaan. Van jongs af aan heb ik interesse voor de natuur, ontstaan rondom de volkstuintjes van mijn ouders, de vele wandelingen en tijdens de vakanties. In 1990 ben ik daarom begonnen met de studie biologie aan de Katholieke Universiteit Nijmegen. Om mijn kennis te verbreden en meer op de praktijk te richten heb ik aanvullend de bovenbouwstudie Natuurwetenschappelijke Milieukunde gedaan. In 1996 heb ik beide studies afgerond. Tijdens mijn studie ging mijn interesse vooral naar waterplanten. Mijn stages richtten zich op de nutriëntendynamiek in een Indonesisch zeegrasveld en op het effect van het toevoegen van ijzer op eutrofiëring en waterplanten in het Nederlandse zoete water. Als laatste stage wilde ik graag bij een onderzoeksinstituut aan de slag. Zodoende kwam ik bij het team Aquatische Ecologie van het toenmalige Instituut voor Bos- en Natuurbeheer in Leersum terecht.

Daar kreeg ik vervolgens mijn eerste baan voor 4 maanden. Na een paar jaar kreeg ik een vaste aanstelling. Weliswaar kreeg ik nauwelijks nog een plant te zien maar ook de macrofauna bleek een interessant studie-object te zijn. Binnen ons team speelde het bouwen van instrumenten voor beoordeling van de kwaliteit van oppervlaktewateren met macrofauna een grote rol. Hierover heb ik dan ook vele projecten uitgevoerd waardoor ik veel kennis opdeed over de ecologie van deze zeer diverse groep van waterbeestjes en niet te vergeten het ontwikkelen van beoordelingsmethoden. Over dat laatste gaat dit proefschrift. Na verloop van tijd is steeds meer gebleken dat we toch terug moeten naar het uitvoeren van fundamenteel onderzoek en uitbreiding van onze ecologische kennis over soorten om vragen vanuit natuur- en waterbeheer echt te kunnen oplossen. Daarom richt ik mij tegenwoordig meer op het uitvoeren van experimenteel onderzoek op ecosysteemniveau waarbij zowel waterplanten als macrofauna een rol spelen.